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MALE GENITAL TRACT OF THE INDIAN AND CEYLONESE PALM SQUIRRELS AND ITS BEARING ON THE SYSTEMATICS OF THE SCIURIDAE

BY

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INTRODUCTION

The value of the male genital tract as one of the criteria for Sciurid classification has been emphasised by POCOCK (1923), MOSSMAN, LAWLAH and BRADLEY (1932), MOSSMAN (1940 and 1953) and PRASAD (1952 and 1954). PRASAD (1954) described the male genital tract of the three striped south Indian palm squirrel *Funambulus palmarum palmarum* Linn; and showed that it differs from the normal type of Sciurid genital tract in a number of features, being characterised by the presence of small intrabulbar Cowper's glands and by the complete absence of a true bulbar gland and glandular penile duct. However SIDDIQUI (1939) described the male genital tract of the five striped palm squirrel occurring in Allahabad, North India, to be of the normal Sciurid type. (He used the name *Funambulus palmarum* incorrectly, as the North Indian five striped palm squirrel is actually *Funambulus pennanti*, WROUGHTON.) He figured well developed Cowper's glands, a bulbar gland and a glandular penile duct joining the urethra outside the musculature of the bulb. His figures and descriptions are not clear. In view of divergences of such a fundamental nature between forms now considered members of the same genus, a comparative study of the male genital tracts of the palm squirrels from different parts of India and Ceylon was undertaken.

The male genital tract of the American red squirrel *Tamiasciurus hudsonicus* described by MOSSMAN et al (1932) resembles that of *Funambulus palmarum palmarum*. In view of this it was thought that the two forms may represent a common line in the evolution of the Sciurid groups.

MATERIAL AND METHODS

Palm squirrels were collected from representative parts of India and Ceylon. The five striped north Indian palm squirrel *Funambulus pennanti* was collected

from Jodhpur, Baroda and Dharbhanga. *Funambulus palmarum favonicus, brodiei* and *kelaarti* were obtained from the National Museums, Colombo, Ceylon and were collected in different parts of the island. The author's description of the genital tract of *Funambulus palmarum palmarum* was verified with a few more specimens collected from different areas in Mysore State (see Fig. 10). As soon as the animals were killed, 10 % formalin or a formol-alcohol-acetic acid mixture was injected into the abdomen and the specimens were wrapped in cotton soaked in 10 % formalin and despatched to the author. Upon arrival at the laboratory the genital tracts were dissected free of fat and connective tissue and preserved in 70 % alcohol. Following the usual procedure of microtechnique, sections of the different regions were cut and stained with Ehrlich's haematoxylin or R. A. Groat's tetrachrome stain. The penes were stained in toto with alizarin and cleared to demonstrate the bacula.

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OBSERVATIONS

Funambulus pennanti (from Baroda, Jodhpur and Dharbhanga-North India).

The male genital tract of this species collected from Allahabad was described by SIDDIQUI (1939) under the incorrect name *Funambulus palmarum*. The genital tract of Siddiqui's *Funambulus palmarum* differs in many features from the type seen in *Funambulus palmarum palmarum*. These differences have been discussed by PRASAD (1954). Detailed study of Siddiqui's figures and descriptions and their comparison with the material studied now on the same species collected from the same areas clearly shows that Siddiqui's identification of the bulbar gland and penile duct in the specimens studied by him were not correct. In view of these discrepancies in his descriptions, a complete redescription is given here, based on specimens collected by the author. The general appearance of the tract is the same as that of *F. p. palmarum* (see PRASAD 1954 for Figures).

Testis. The testes are scrotal in sexually mature animals and are never found retracted into the abdomen, even in dead animals. The thin tunica albuginea is ornamented by a series of 5—7 parallel veins forming well marked longitudinal stripes. The testes reach a maximum size of 18×14 mm. The epididymis is closely applied to the testis.

Prostate. The prostate gland forms an encapsulated mass the ventral surface of which is flat while the dorsal is grooved medially for the accommodation of the rectum. The cephalic end is smoothly rounded but the caudal end is cleft in the middle for the lodgement of the muscular urethra. The gland lies completely outside the urethra. The ducts of the different lobules converge towards the caudal end of the gland where they unite to form a pair of common ducts

which continue backwards to pierce the roof of the muscular urethra and run through the colliculus seminalis.

Seminal vesicles. The seminal vesicles are smaller than in *F.p.palmarum.* These are highly branched tubular glands held together by a thin and easily separable membranous fascia to form a mass of irregular contours. The two main lobes of each gland drain by separate ducts which unite at the caudal end and proceed towards the muscular urethra. The seminal vesicle duct, like that of the prostate, pierces the dorsolateral roof of the muscular urethra to enter the colliculus. In the sexually mature animals the gland reaches a maximum size of $6 \times 12 \times 14$ mm in depth, breadth and length respectively.

Muscular urethra. The muscular urethra extending from the prostatic region to the bulb is about 15 mm long. It has a thick wall of circular striated muscle fibres. It differs from that of the south Indian palm squirrel in the presence of well developed mucous folds projecting into the lumen. At the neck of the bladder there is a distinct cephalically directed dorsal outpouching of the urethral lumen. A few millimeters caudal to this fold, also on the dorsal wall of the urethral lumen, are three successive pairs of transverse semilunar folds of the mucosa, the concavities of which are directed cephalically. The ridge formed by the continuation of the fold of the colliculus seminalis along the middorsal line divides these folds into paired structures (Figs. 13 and 14). The colliculus is well developed and broad. The function of the mucous folds is not clear.

The vasa deferentia are most median and are flanked on either side by the ducts of the seminal vesicles. The prostatic ducts are most lateral. All three pairs of genital ducts run along the colliculus seminalis and open into the urethra independently. The utriculus prostaticus (uterus masculinus or vagina masculina) is well developed and in some specimens assumes very large proportions. It opens into the urethra independently (Figs. 13 and 14).

Cowper's glands. The Cowper's glands are completely enclosed in the fibrous capsule of the bulb but they are more extensive and occupy a larger part of the bulb than in the south Indian palm squirrel. The glands expand towards the proximal part and almost fill the entire capsule of the bulb. Each of the glands leads into a well formed glandular duct within the bulb. These two Cowper's ducts converge towards the mid-ventral line and unite at the distal end of the bulb (Fig. 15). This common duct of the Cowper's glands runs distalwards ventral to the urethra completely enclosed in the fibrous capsule of the corpus-spongiosum of the penis until it unites with the urethra at the end of the proximal one third of the penis body (Figs. 9 and 18). The common duct of the Cowper's glands shows no enlargement or modifications within the bulb to form the bulbar gland so uniformly present in the genital tracts of *Sciurus carolinensis, Tamias striatus* (MOSSMAN et al 1932) and *Ratufa indica* (PRASAD 1954). Hence the common glandular duct leading from the bulb ventral

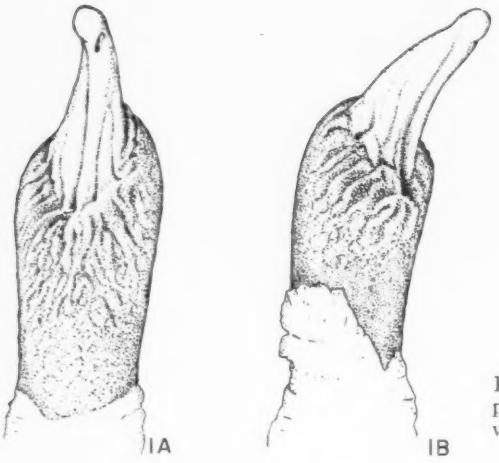

Fig. 1. Glans penis of the north Indian palm squirrel *Funambulus pennanti*. a. ventral view ×5¾; b. lateral view ×5¾.

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to the urethra can only be considered as a continuation of the Cowper's ducts, not as a close homologue of the penile duct of the other squirrels. SIDDIQI (1939) described and figured a well developed bulbar gland and penile duct in his specimens from Allahabad. His figures and descriptions are misleading and apparently inaccurate.

Penis. The penis is about 45 mm long from the base of the bulb to the tip of the glans penis. Sections of the corpus cavernosum in the proximal one-third of the penis body (Fig. 17) show two ducts, a dorsally placed urethra and a ventral common duct of the Cowper's glands. These, as described above, unite at the junction of the proximal one third of the penis body with the beginning of the middle one third (Fig. 18).

Glans penis. The glans penis is smaller than in *F.p.palmarum*. Typical measurements of the adult penis are:

Length of the glans penis: 10 mm, transverse diameter: 5—6 mm, dorso--ventral diameter: 5—6 mm.

POCOCK (1923) described the glans of *Funambulus* as follows "glans ending in a narrow elongated point supported by a distal portion of the baculum. Orifice to the right near the base of the slender portion and remote from the tip. Baculum a simple upcurved attenuated rod". The glans of *Funambulus pennanti* agrees in general with this description. It is cylindrical with a curvature gradually rising from the base towards the extremity (Fig. 1, a and b). The tip of the glans is raised into a pointed elongated prominence which is supported internally by the distal prolongation of the baculum. The dorsal part of the prominence is slightly arched while the ventral shows a slight bulge a few millimeters proximal to the terminal end. The absence of the mucous folds

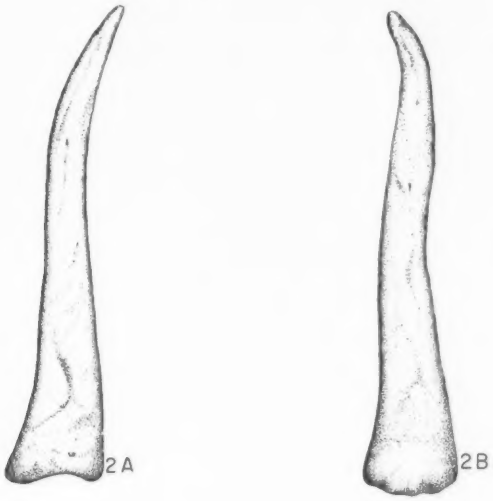

Fig. 2. Baculum of *Funambulus pennanti*. a. ventral view × 6½; b. lateral view × 6½.

on the glans and the pointed projection at the tip distinguish it from all other squirrels of the species *Funambulus palmarum*. The meatus urinarius is in the form of an oblique slit on the right side of the glans near the terminal end on the pointed elevation of the glans.

Baculum. (Fig. 2, a, b). The baculum is a narrow rod 9.5 mm in length. It is broad at the proximal end and narrows to a point terminally. It is curved to conform to the curvature of the glans. The terminal end supports the pointed elevation at the tip of the glans. There are no traces of the bifurcation of the tip so characteristic of *F.p.palmarum*.

Funambulus palmarum kelaarti (from Ceylon)

In describing the male genital tracts of the Ceylonese palm squirrels reference will be made only to variations from the condition seen in *Funambulus palmarum palmarum* Linn, described by PRASAD (1954).

The three pairs of genital ducts run through the well developed colliculus seminalis. The vasa deferentia are most medial, on either side are the ducts of the seminal vesicles. A few millimeters before opening into the urethra the prostatic ducts which lie lateral to those of the seminal vesicles bend inward and unite with the seminal vesicle duct of the corresponding side and open into the urethra by a common duct. The vasa deferentia open independently. In one specimen out of four, a series of three pouches on the dorsal wall of the muscular urethra was well developed as in the case of *Funambulus pennanti*. This is only an individual variation.

Cowper's glands. The Cowper's glands are the least developed of the accessory

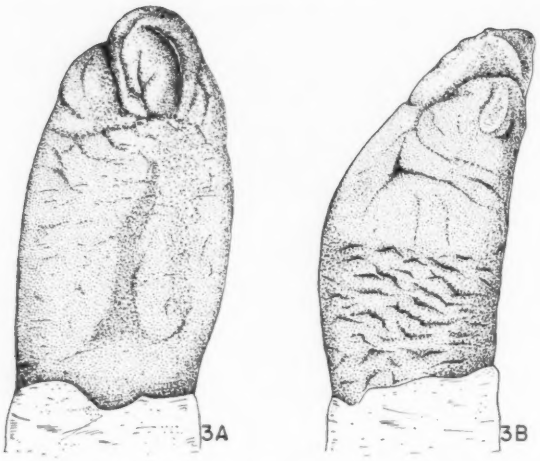

Fig. 3. Glans penis of the Ceylonese palm squirrel *Funambulus palmarum kelaarti*. a. ventral view × 4 ½; b. lateral view × 4 ½.

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glands and are enclosed in the fibrous capsule of the bulb. There is no trace of either a bulbar gland or penile duct (Fig. 16).

Glans penis. The glans penis of this subspecies has not been described earlier. It is cylindrical, smooth proximally and terminates in an upwardly directed blunt projection (Fig. 3, a, b). The ventral bulge of the glans is prominent. The dorsal margin of the glans is almost straight. The distal end is conical and is faintly demarcated into two lobes, the left being slightly larger and more rounded than the right. The opening of the urethra is towards the right on the right half of the conical projection. The base and body of the glans are sculptured by a series of small transverse fissures. A small groove running across the glans from the base to the right lobe represents the fold of mucous membrane so characteristically seen in the glans of *F.p.palmarum*.

Baculum. (Fig. 4, a, b,). The baculum is a rod about 13 mm in length, flattened laterally. It is slightly concave on the dorsal side from the proximal to the distal extremity. Terminally the bone is clearly bifurcated. The right limb of the fork is longer and bent with the concavity towards the tip of the glans, while the left limb is in the form of a blunt protuberance on the shaft. The right limb of the baculum supports the raised prominence on the right side of the glans. The urethra runs asymmetrically to the right of the larger limb and opens a few millimeters from the terminal end.

Funambulus palmarum favonicus and *F. p. brodiei* (from Ceylon)

The genital ducts have the same course in the colliculus seminalis as in *F.p. palmarum*.

Fig. 4. Baculum of *Funambulus palmarum kelaarti*. a. dorsal view × 5; b. laterals view × 5.

Cowper's glands. The Cowper's glands are least developed in these. The glandular patches are enclosed within the fibrous capsule of the bulb and their ducts are short and open into the urethra within the bulb (Fig. 16).

Seminal vesicles. The seminal vesicles are noticeably larger than in the other palm squirrels. In the sexually mature animals they attain a size of 9 × 16 × 18 mm in depth, breadth and length respectively.

Glans penis. Although the two subspecies are closely similar in the other parts of the genital tract, differences are noticeable in the glans penis and baculum (see HILL 1936 for figures). HILL gives the following measurements of the glans and baculum of these subspecies:

Subspecies.	Length of glans	Transverse diameter of glans	Dorso-ventral diameter of the glans	Length of baculum
favonicus	12 mm	6 mm	7 mm	12.5 mm
brodiei	13 mm	5 mm	7 mm	12.0 mm

The description of the glans and baculum of these forms is taken from HILL (1936) to which reference can be made for the figures.

"The glans penis in both forms consists of a proximal smooth cylindrical portion and an apical more globular part. The two parts are separated by a faintly constricted neck, which in *brodiei* is better seen on account of a deposit of melanin pigment ventrally and at the sides.

The globular portion is truncated distally, but more so from the right than the left, so that, when viewed from above or below, the left upper portion of

the apex forms a rounded summit caused by the presence of the tip of the baculum under the mucosa. The meatus urinarius is situated to the right and below this prominence, and lies within one of the cleft-like fissures into which this part of the glans is sculptured. In *favonicus* there are four or five small fissures below the meatus urinarius passing obliquely from above and to the left downwards and to the right. Another deeper fissure lies at the bases of these and at right angles to them. Other smaller fissures are seen further back, especially on the left side. Fissuration is less evident on the glans of *brodici*.

Baculum. The baculum in *favonicus* and *brodici* is an attenuated slightly curved rod, flattened laterally. In both forms it ends in a bifurcated manner, but the exact form of the end is slightly different in the two races. In *favonicus* the lower limb of the fork is just the enlarged distal end of the shaft. The upper limb, which may form a separate ossicle, is a pointed process responsible for the prominence already mentioned at the tip of the glans.

In *brodici* the baculum is more distinctly bifid at the tip. The lower limb of the bifurcation is short and rounded. The upper is narrow, long and hooked at the tip, the dorsum of the hook being responsible for the superficial prominence of the glans. The shaft of the baculum in both races is markedly asymmetrical when viewed from above or below. It is concave on the right where it lodges the urethra, and convex on the left".

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DISCUSSION

The prevailing scheme of classification of the Sciuridae is based to a large extent on characters of pelage, teeth and skull and to a minor degree on the male genital tract in some cases. MOSSMAN (1953), points out that the most generally accepted basis for classification is phylogeny, that is, the evolutionary or genetic relationships within the group. In such a system the more conservative characters (those least subject to evolutionary modification due to selective adaptation) may be of use in characterising higher categories while the less conservative ones will be of use in differentiating the lower categories. The structural characters of the reproductive system show relatively little evolutionary divergence since they are largely independent of environmental selection. In summarising his arguments MOSSMAN says "such an environmentally isolated system must then be very conservative in its characters as compared to those organ systems related closely to the environment and therefore subject to intense adaptive evolution. The characters of such a system should vary relatively little between major groups, and almost none between closely related minor categories. This lack of divergence should make it possible to detect common characteristics among groups widely separated from one another in other characteristics, whether this be due to adaptations to divergent environments, or to

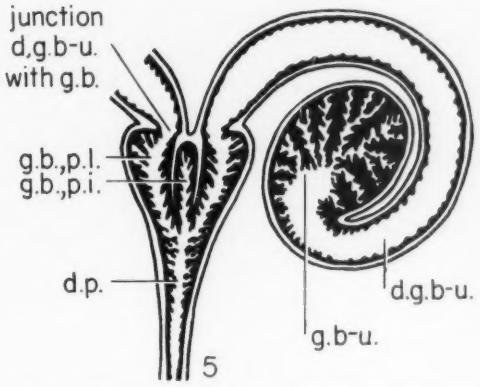

Fig. 5. Diagrammatic section through the bulb and one Cowper's gland of the *PENILE DUCT* type of genital tract showing the bulbar gland and penile duct.

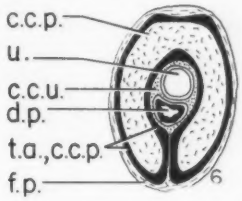

Fig. 6. Diagrammatic section through the body of the penis of the *PENILE DUCT* type of genital tract showing the penile duct and the urethra.

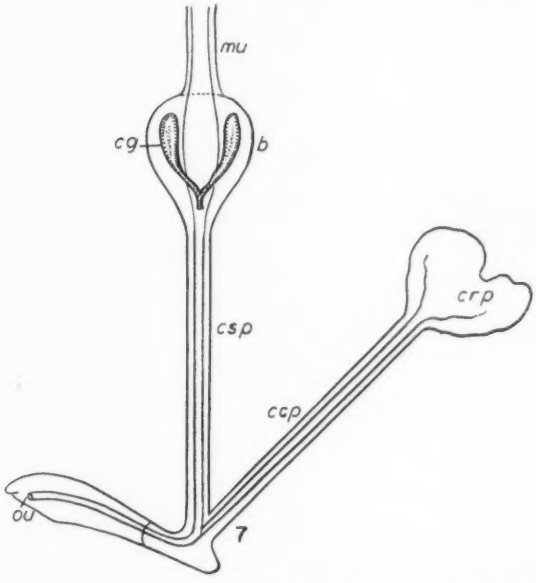

Fig. 7. Diagram of the penis of the *NO PENILE DUCT* type of genital tract showing the position of the Cowper's glands, its duct and the urethra.

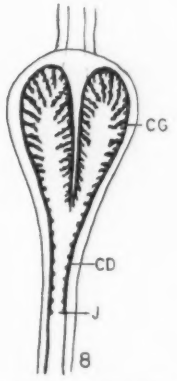

Fig. 8. Diagrammatic section through the bulb of *Funambulus pennanti* showing the extent of the Cowper's glands in the bulb. The Cowper's duct unites with the urethra at the end of the proximal one third of the penis body.

Fig. 9. Diagrammatic section through the body of the penis of the *NO PENILE DUCT* type of genital tract showing the absence of the penile duct.

Fig. 10. Map of India showing the different places where the squirrels were collected for this study. Scale 1 inch = 200 miles.

extinction of intergrading forms. Conversely, dissimilarity in characters of a conservative organ system should be good evidence for lack of close phylogenetic relationship".

The male genital tract of the Sciuridae lends itself admirably to the elucidation of these points. MOSSMAN et al (1932) described the male genital tract of a number of North American squirrels and pointed out that the typical Sciurid male genital tract (Figs. 5, 6, 11 and 19) is characterised by the presence of well developed large Cowper's glands leading into the bulb by wide spiral ducts which constrict as they enter the bulb, but on entering it expand and then unite

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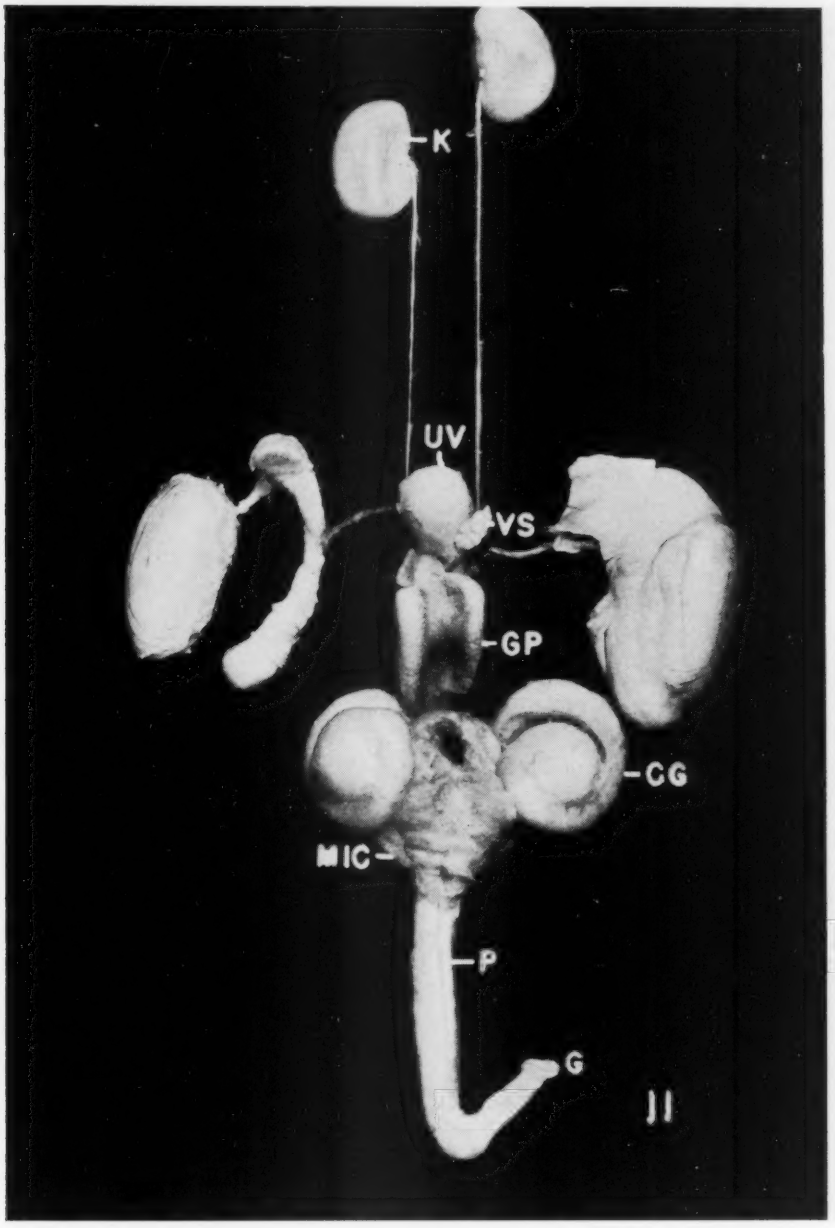

Fig. 11. Dorsal view of the uro-genital tract of the *PENILE DUCT* type seen in the gray squirrel *Sciurus carolinensis*. The right epididymis has been separated to show its parts and the ductuli efferentia.

to form at the region of union a well developed, bulbar gland. The bulbar gland drains through a glandular penile duct which runs ventral to the urethra in the corpus spongiosum of the penis and unites with it near the ventral flexure. The glans penis and baculum are well developed. This type of genital tract will be designated in this paper—the *PENILE DUCT TYPE*. The penile duct type is characteristic of the large majority of the squirrels so far studied. The American red squirrel *Tamiasciurus hudsonicus* and the Indian palm squirrel *Funambulus palmarum palmarum* differ from this pattern in the absence of Cowper's glands outside the bulb, and in the absence of a bulbar gland and glandular penile duct leading from it (Figs. 7, 8, 9 and 12). This type of genital tract will be designated—the *NO PENILE DUCT TYPE*. There is in *Tamiasciurus* a non-glandular ventral diverticulum or sinus opening into the urethra in the bulb. This sinus is, however, absent in the penile duct type of genital tract as well as in the palm squirrels. Pocock (1923) separated *Tamiasciurus* from the genus *Sciurus* on account of the peculiar filamentous nature of the glans penis and the supposed absence of the baculum. The studies of Mossman et al (1932) substantiated Pocock's view showing that the male genital tract is of classificatory value. Layne (1952) demonstrated the presence of a minute baculum in this species.

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While the male genital tract shows certain characters which can be applied to advantage in determining phylogenetic relationships of the Sciuridae, a summary of the foetal membrane characters of the Sciuridae (Mossman 1937, Mossman and Weisfeldt 1939 and Veeriah 1944)—Table I, shows that they are essentially the same in all squirrels studied; so similar that they cannot be readily used in evaluating intergeneric differences within the group. In other words they are an even more conservative group of characters than those of the male genital tract.

The Indian palm squirrels *Funambulus palmarum palmarum* (Prasad 1954), *Funambulus palmarum kelaarti, favonicus, brodici* and *Funambulus pennanti* have a *no penile duct type* of genital tract. These have a remarkable resemblance to the condition in *Tamiasciurus*, but lack the urethral sinus and have a much stouter glans penis and a large baculum. The Indian Malabar squirrel *Ratufa indica maxima* has the *penile duct type* of genital tract (Prasad 1954 for figures).

Ellerman (1940 and 1946) and Simpson (1945) include under the family: *Sciurinae*, tribe: *Funambulini*, the genera *Funambulus* and *Ratufa*. Since the value of the male genital tract as a criterion for Sciurid classification seems to be sufficiently well established, it seems unreasonable that these two genera showing two distinctly different types of male genital tracts should be included in the same tribe with each other. Describing the penis and os penis of Ceylonese squirrels Osman Hill (1946) says "there is no doubt about the apartness of *Ratufa* from other genera (*Funambulus* and *Tamiodes*) in the nature of its

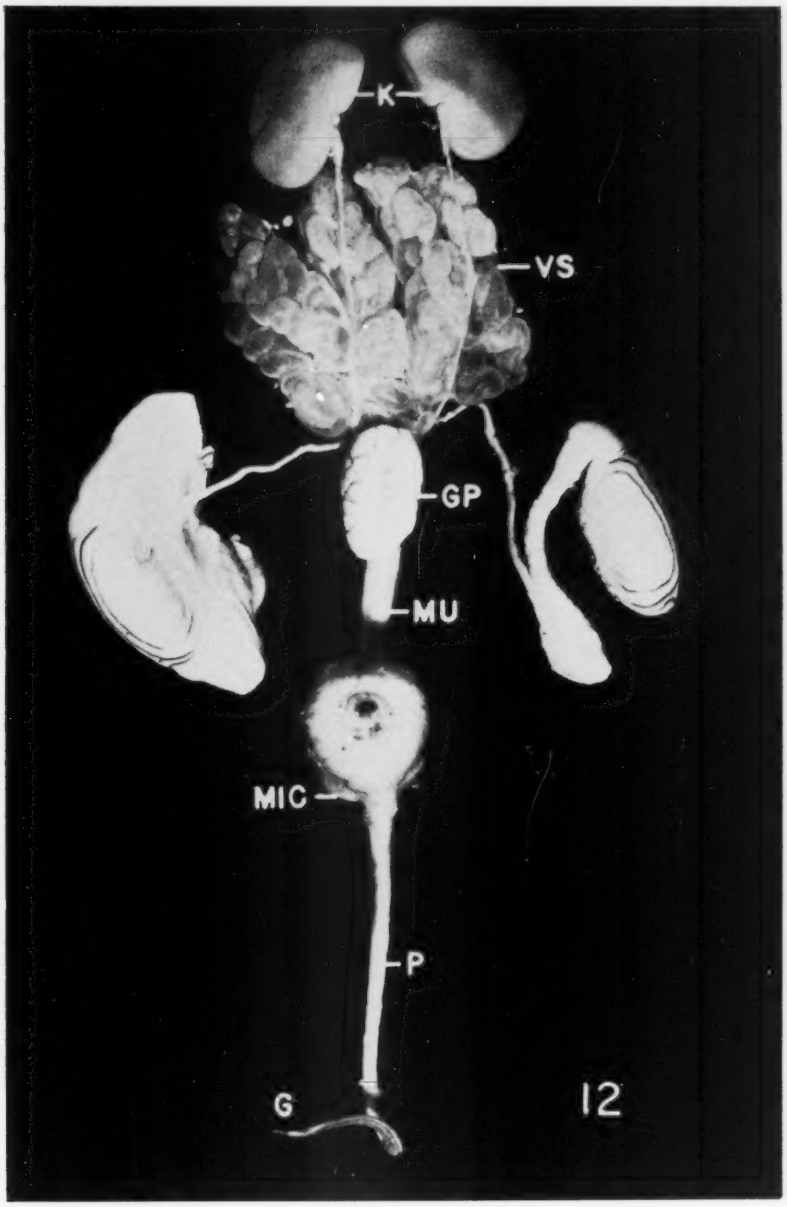

Fig. 12. The uro-genital tract of the *NO PENILE DUCT* type seen in the American red squirrel *Tamiasciurus hudsonicus*. Dorsal view. Note the absence of the typical large Cowper's glands and also the long filiform penis.

TABLE I

Summary of the foetal membrane characters of a few members of the Sciuridae.

	Implantation	*Amniogenesis*	*Yolk sac*	*Chorio-allantoic placenta*	*Chorio-vitelline placenta*
PENILE DUCT TYPE of male genital tract. *Sciurus carolinensis* *Sciurus niger* *Tamias striatus* *Citellus 13 lineatus*	Orientation (Disc): Mesometrial First attachment: Antimesometrial Depth: Superficial but eccentric	Folding	Bilaminar omphalopleure: large. persists till term Trophoblast gives rise to mononuclear giant cells	Shape: Discoid Type: Labyrinthine Finer morphology: haemochorial Location: Mesometrial Allantoic vesicle: small and permanent	Well developed. persists till limbbud stage. Vascular splanchnopleure: incomplete inversion, small. Non villous
Ratufa indica (details of embryology not studied)	(The number of attachment cones are slightly variable)				
NO PENILE DUCT TYPE of male genital tract					
Tamiasciurus hudsonicus *Funambulus palmarum palmarum*	Orientation: Disc: Mesometrial First attachment: Antimesometrial	Folding	same as above in all essential features		
F.p.favonicus *F.p.kelaarti* *F.p.brodiei* *F.pennanti* (Details of embryology not studied)	Depth: Superficial but eccentric Attachment cones variable				

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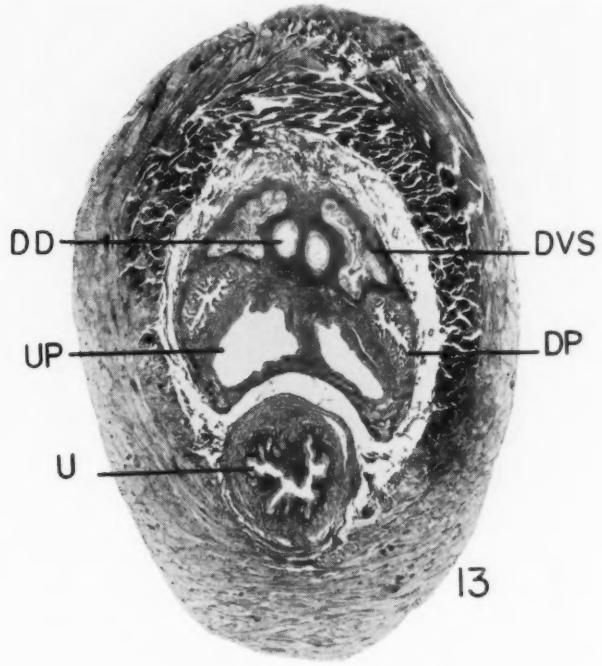

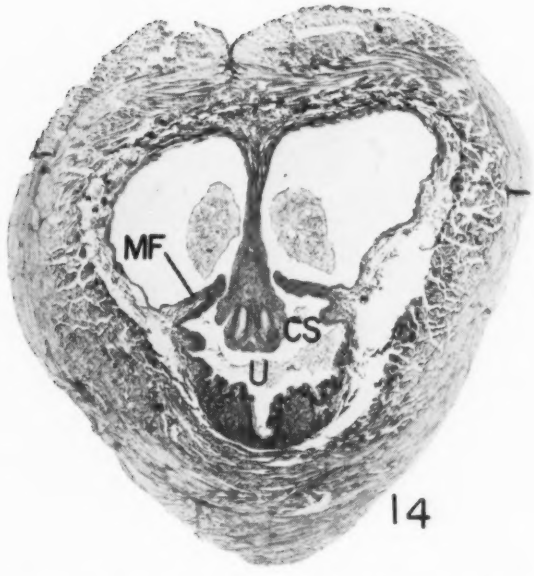

Figs. 13 and 14. Cross sections through the muscular urethra of *Funambulus pennanti* showing the course of the genital ducts and the muscular folds.

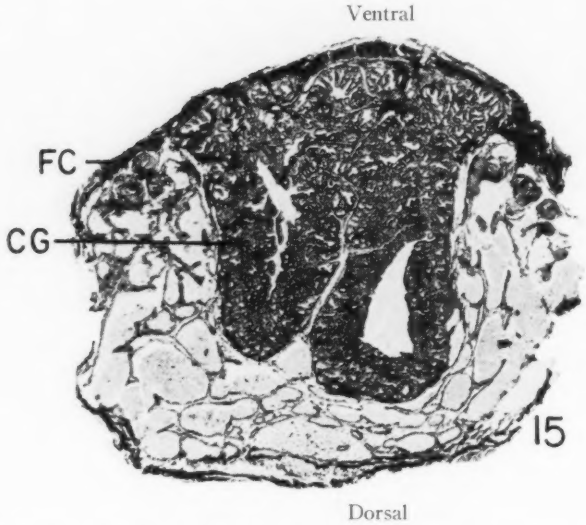

Fig. 15. Cross section through the bulb of *Funambulus pennanti* showing the intra-bulbar Cowper's glands more extensive than in south Indian and Ceylonese palm squirrels. From the syphysis pubis the penis passes forward along the mid-ventral body wall. The position of the Cowper's gland and its duct is *Ventral* and that of the urethra *Dorsal*. Photographs 15—19 are oriented with the urethra below.

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baculum. The nature of the glans confirms this." Prasad's studies on the male genital tract of *Ratufa* substantiate this conclusion. Osman Hill (1940) describes the penis and os penis of *Ratufa macroura melanochra* which differs in many respects from that of *Ratufa macroura sinhala*. Considering these diferences he favours the old view of giving them full specific status. He further remarks "... obviously we are dealing with forms which have evolved much further and have become much more completely differentiated than most mammalian species have from one another ...". These observations clearly show that in the absence of any other distinctive character, even such minor characters as the appearance of the glans and baculum can be made use of in differentiating subspecies.

In view of the foregoing discussion it is suggested that the genus *Funambulus* be shifted from its present position and that all other genera of squirrels now included under the tribe *Funambulini* in Simpson's classification be tentatively classed under the tribe *Sciurini* till their position can be definitely assigned by a study of their male genital tracts. It seems best to retain *Funambulini* solely for the genus *Funambulus*. Pocock (1923) included under the subfamily *Funambulinae* a number of squirrels which were highly diversified. He considered this group as a "highly diversified group of genera with glans exceedingly variable in size and structure and baculum either relatively very large (*Funambulus* and *Tamiodes*), relatively small (*Protoxerus and Ratufa*), minute

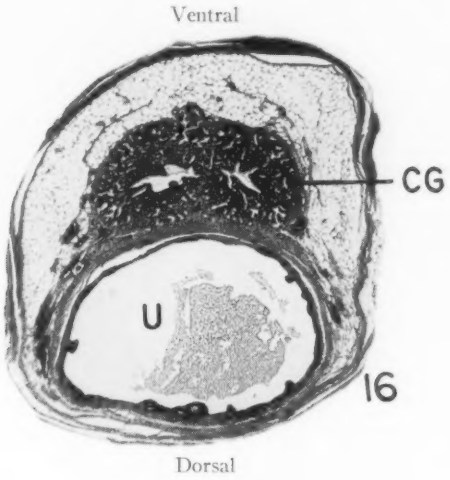

Fig. 16. Cross section of the bulb of the south Indian and Ceylonese palm squirrels showing the small sized intra-bulbar Cowper's glands. Compare Fig. 15.

(*Funisciurus, Paraxerus, Aethosciurus*) or absent (*Heliosciurus*)". *Ratufa* has been shown to possess a large baculum. It would be of great interest to study the male genital tract of a number of South African genera of squirrels in which the baculum is known to be minute or absent. Like *Tamiasciurus* their genital tracts may turn out to be extremely interesting. Till a final study of these genera is made, they will be tentatively accommodated under the tribe *Sciurini*. Attempts are being made to obtain these South African squirrels.

It also seems reasonable to question the validity or logic of placing *Tamiasciurus* and *Funambulus* as tribes of the subfamily *Sciurinae* when their reproductive tracts are so different from the other members of the group. MOSSMAN (1953) remarked in this connection "... but *Funambulus* is like *Tamiasciurus* in having minute Cowper's glands and no bulbar gland and penile duct. Obviously this indicates close relationship between this species and *Tamiasciurini*, and poses a number of interesting questions in regard to Sciurid phylogeny and geographical distribution, to say nothing of the doubt that it throws on the taxonomy of the whole group". This observation leads one to the assumption that the Sciuridae may consist of two different and divergent stocks, one represented by the *penile duct type* and the other by the *no penile duct type*. It will be interesting to investigate the possibility of intergrading forms between the two. If such intergrading series do exist the genital tracts are not so distinctive in classification as they appear at present.

It was for this reason that the author made this study of the genus *Funambulus* from all parts of India and Ceylon. There are three groups in this genus, namely, the *palmarum* group including the species *pennanti, tristriatus* and *palmarum*; the *layardi* group including the species *layardi*; and the *sublineatus* group including the species *sublineatus*. *Funambulus palmarum* with its different

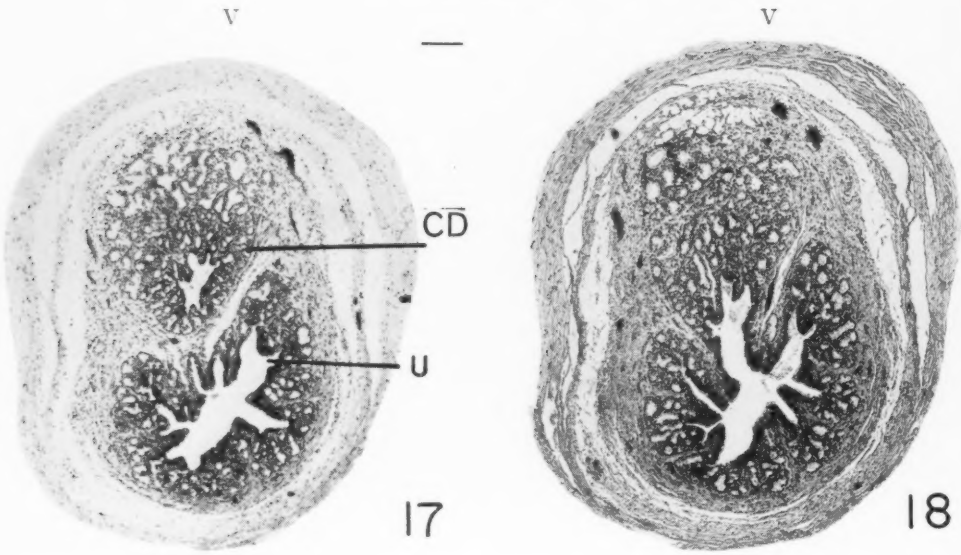

Fig. 17 and 18. Cross sections of the penis of the north Indian palm squirrel *Funambulus pennanti*. Fig. 17 shows the sections passing through the proximal one third of the penis where the common Cowper's duct and the urethra can be seen. Fig. 18 shows a section passing at the end of the proximal one third of the penis body where the Cowper's duct is opening into the urethra.

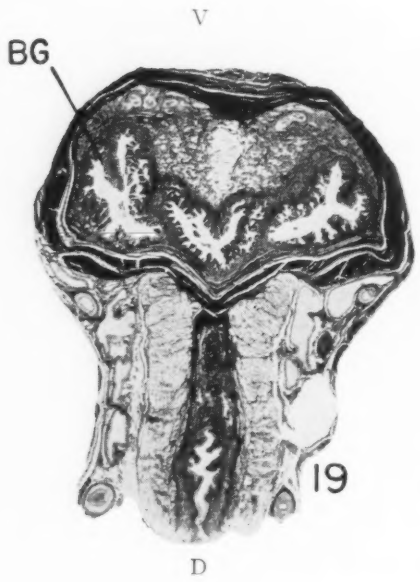

Fig. 19. Section passing through the bulb of the Penile duct type of genital tract seen in *Sciurus carolinensis*. The bulbar gland is clearly seen.

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subspecies is the common three striped palm squirrel distributed in most parts of south India and Ceylon. The Pennant's five striped palm squirrel *Funambulus pennanti* is the commonest form in all parts of north and western India and in some parts of the Bombay Presidency. The species *Funambulus tristriatus* also occurs in north India and in some parts of south and western India north of Bombay. The palm squirrel in Ceylon is represented by the species *palmarum* with its four named races *favonicus, brodiei, kelaarti* and *olympius*. Of the jungle squirrels two species are recognised namely *layardi* and *sublineatus*, each with a single subspecies. OSMAN HILL (1936) described the penis and os penis of the Ceylonese palm squirrels and pointed out specific and subspecific differences. The author has now studied the male genital tracts of *Funambulus palmarum palmarum* from south India, *Funambulus pennanti* from three widely separated areas in North India, namely Baroda, Jodhpur and Dharbhanga and *Funambulus palmarum favonicus, brodiei* and *kelaarti* from Ceylon. Geographically (Fig. 10) these forms represent fairly well all parts of India and Ceylon.

The classification of the Sciuridae followed by SIMPSON is based to some extent on POCOCK (1923) who classified the group on the nature of the baculum and the glans. In Simpson's classification a few of Pocock's families have been reduced to the rank of subfamilies and tribes. Summarising his view on this question Simpson remarks "Differences between the 'orthodox' method and 'reproductive system' method of classification of Sciuridae are especially noticeable on two points. 'Orthodox' students of classification (eg. THOMAS, MILLER and GIDLEY) recognise a subfamily Nannosciurinae for Nannosciurus and Sciurillus... POCOCK claims in a way convincing to me that these are independent forms in three widely separated areas, convergent in small size and related habitus characters but not especially related. Orthodox students place *Tamiasciurus* as, at most, a subgenus of *Sciurus*. POCOCK showed that its reproductive system is wholly unique and made a separate subfamily for it. This was confirmed by MOSSMAN, LAWLAH and BRADLEY who incline to remove it from the Sciuridae altogether, but they are not taxonomists and this is really extreme. The distinction is here tentatively given a tribal value". ELLERMAN (1940) is not in favour of a classification based on genital tract characters and remarks "... But if this character is given such importance, I fail to see how fossil forms are to be considered; and it seems that if cranial and dental characters have been used primarily for classification since the days of Linnaeus one cannot be blamed for wishing to continue to give more importance to these characters than to an external character which has only been definitely verified in a very small percentage of named species and races, and found to be subspecifically variable in at least one case".

There is no order of mammals where there are so many uncertainties in current classification as there are in rodents. The Sciuridae are a large and

complex group and it would be hazardous to base the classification solely on one set of characters. The most generally accepted basis of classification is phylogeny—the evolutionary and genetic relationships within the group. The dental and skeletal characters are of great value in the classification of the fossils. In so far as the living genera are concerned it is emphasised that adequate consideration should be given to genital tract characters which have been shown to be conservative and whose value as one of the criteria for systematics has been proved beyond question. Inspite of the hesitation of the 'orthodox' system of classification to accept genital tract characters for taxonomy of the Sciuridae, on the basis of earlier discussion and in view of the extreme divergence in the nature of the *no penile duct type* of genital tract possessed by *Tamiasciurus* and *Funambulus* there seems to be valid reason for shifting them from their present position wherein they have been placed with forms showing the *penile duct type* of genital tract.

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The separation of these two genera from the rest of the Sciuridae brings up the important question of the relation of geographic distribution to classification. The two genera are so widely separated geographically that there are at present no known forms which can be shown to be intermediate in linking them. They differ appreciably in skeletal and dental characters (see Appendix) but show a marked similarity in genital tract characters. Dr. A. E. Wood in a personal communication says that palaeontological evidence in so far as the Sciuridae are concerned is very scanty and scattered and throws no light on the affinities of these two genera with others. It is possible that there may have been extensive and vast migrations of either of these two genera or their ancestral types from one continent to the other. The recent Sciuridae are a very widely diversified group, highly adapted to different environments. In such a plastic group it is possible that Tamiasciurus like forms migrated across the land bridge near the Behring strait onto the northeastern tip of Asia in the remote past and gradually moved south across the mainland of China into India. Palaeontological evidence of such migrations among Sciuridae is lacking.

The geology of the Himalayas shows that the first uplift occurred in the middle of the Eocene and continued until the Pleistocene. Since then the Himalayan ranges have formed an almost impassable barrier between the mainland of China and India. Dr A. E. Wood (personal communication) says "the uplift of the Himalayas did not give a continuous barrier, since there were long periods of erosion. It could easily have been possible for tree living forms to cross that area in the Oligocene or Miocene. At present we know nothing of the origin or intercontinental migrations of squirrels as a whole or their component groups. It would therefore be possible that migrations could have been in almost any direction at any time from any place". As far as the author is aware there are no indications of any forms close to *Tamiasciurus* outside North America or of forms close to *Funambulus* outside southern Asia. In the absence of any other

evidence showing close relationship between these two genera, except similarities of the male genital tracts, the only course open is to suggest that the evolution of these two genera in the direction of the *NO PENILE DUCT* type may have been independently along parallel lines, one in the Nearctic leading to *Tamiasciurus* and the other in the oriental region leading to *Funambulus*. In this connection it might be mentioned that there are a number of genera of Sciurids which have evidently migrated between Northern Asia and North America (*Marmota, Citellus, Eutamias*), and are interrelated.

Among rodents parallelism and convergence have been the main evolutionary trends within the group superimposed on the basic divergence of the rodents from the other mammalian stocks. The specialisation of the ever growing incisors and associated changes in the teeth led to a restricted adaptive radiation resulting in a number of independent lines, which have continued in the same general direction. As a result there is parallelism. The most striking case of parallelism is shown by the old world and the new world porcupines belonging the Hystricomorpha. Wood (1937, 1947 and 1950) gives a number of instances of parallelism, which he terms "parallel radiation", in different groups of rodents and discusses their evolutionary significance.

The extent of parallelism within the family Sciuridae in a conservative organ system like that of the male genital tract must be of evolutionary significance. The degree of such a parallelism can only be determined after a detailed and extensive study of the male genital tracts of all the South African, South American and Asiatic squirrels. This study will be taken up as soon as sufficient material is available.

Since *Tamiasciurus* and *Funambulus* have certain dissimilarities of skeleton and dentition, since no intergrading forms are known, and in view of the wide geographical separation between them, it is suggested that inspite of their similarities in genital tracts, each genus should be elevated to a separate subfamily—*Tamiasciurinae* and *Funambulinae*. In view of the foregoing discussion it is emphasised that in the systematics of the Sciuridae strong consideration should be given to genital tract characters as well as dental and skeletal characters. Reliance should not be placed solely on any one of them.

Accordingly the following classification of the Sciuridae, as modified from Simpson (1945—page 78) is proposed. Only those subfamilies and genera which are affected by this schema are included here.

Family. *Sciuridae*.

Subfamily.	*Tamiasciurinae*.	Example. *Tamiasciurus*.
Subfamily.	*Funambulinae*.	Example. *Funambulus*.
Subfamily.	*Sciurinae*.	

Tribe. *Sciurini.* Examples. *Sciurus, Neosciurus* etc.
Under this tribe will be included all genera except *Funambulus* which were, in Simpson's classification, included under the tribe *Funambulini.* These genera are *Ratufa, Protoxerus, Epixerus, Funisciurus, Paraxerus, Heliosciurus* and *Myosciurus.*

(the rest of the classification is unaffected by this scheme)

The characters of the two subfamilies Tamiasciurinae and Funambulinae are given in Appendix I.

SUMMARY

The male genital tract of the palm squirrels of India and Ceylon has been studied with a view to ascertain if characters of sufficient importance could be seen to assess their interrelationships within the Sciuridae. *Funambulus pennanti* collected from three widely separated areas in North India, *Funambulus palmarum palmarum* from South India, and *Funambulus palmarum favonicus, brodiei* and *kelaarti* from Ceylon have been compared.

The main point of interest concerns the nature of the Cowper's gland and its duct. In all the palm squirrels examined Cowper's glands are completely enclosed in the fibrous capsule of the bulb and there is no trace of a bulbar gland or penile duct. This resembles closely the condition seen in the American red squirrel *Tamiasciurus hudsonicus.* This type of genital tract is designated the *NO PENILE DUCT TYPE.* In Ceylonese squirrels the Cowper's glands are smaller in size and the Cowper's duct is also short and enters the urethra within the bulb. *Funambulus pennanti* has slightly larger Cowper's glands which extend through a larger part of the bulb. The common duct of the Cowper's gland proceeds distalwards ventral to the urethra enclosed in the corpus spongiosum of the penis and unites with it at the end of the proximal one third of the penis body.

The genital tract of the other Sciurid genera show large Cowper's glands outside the bulb and well developed bulbar gland and glandular penile duct which runs through almost the entire length of the penis body and unites with the urethra near the ventral flexure. This type of genital tract seen in *Sciurus, Citellus, Ratufa* etc is designated the *PENILE DUCT TYPE.*

It is suggested that the *penile duct type* and the *no penile duct type* of genital tract may represent two different and divergent lines of Sciurid evolution. On the basis of this assumption a modification of the prevailing scheme of Sciurid classification is suggested.

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In Simpson's classification *Funambulus* and *Ratufa* which show two different types of genital tracts are included in the same tribe *Funambulini*, under the subfamily *Sciurinae*. On the basis of the author's present studies it seems that *Funambulus* should be separated from its present position and elevated to the rank of a subfamily. Likewise *Tamiasciurus* should be elevated to the rank of a subfamily. Until genital tract studies are made, all genera now included under the tribe *Funambulini* in Simpson's classification should be included tentatively under the *Sciurini*.

It is emphasised that for purposes of Sciurid classification strong consideration should be given to genital tract characters as well as to dental and skeletal characters.

The evolution of the *No penile duct type* may have been along parallel lines, one in the Nearctic leading to *Tamiasciurus* and the other in the oriental region leading to *Funambulus*.

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KEY TO FIGURES 1—19

BG.	Bulbar gland	GBU	Glandulae bulbo-urethralis. (Cowper's gland)
B.	Bulb	GP	Glandulae prostaticae
CCP	Corpus cavernosum penis	J	Junction of the common Cowper's duct with the urethra
CCU	Corpus cavernosum urethrae	K	Kidney
CD	Common Cowper's duct	MIC	Musculo-ischio-cavernosus
CG	Cowper's gland	MU	Muscular urethra
Cr P	Crus penis	MF	Muscular fold in the muscular urethra
CS	Colliculus seminalis	OU	Opening of the urethra
DD	Ductus deferens	ta CCP	Tunica albuginea of Corpus cavernosum penis
D	Dorsal	U	Urethra
DP	Penile duct	UV	Urinary vesicle
DG BU	Ductus glandulae bulbo-urethralis. (Duct of the Cowper's gland)	VS	Vesicula seminalis
DVS	Ductus vesicula seminalis	V	Ventral
FP	Fascia penis		
FC	Fibrous capsule		
GB	Glandula bulbi (Bulbar gland)		

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APPENDIX I

Characters of the subfamilies *Tamiasciurinae* and *Funambulinae*. Dental and skeletal characters given here have been taken verbatim from "Families and genera of living rodents" by J. R. Ellerman, British Museum of Natural History, London. 1940.

Funambulinae

Range: India, Ceylon, and Baluchisthan.

Characters:— Skull with more or less elongate rostrum; postorbital process not large; parietal ridges frequently joined together. Zygomatic plate as a rule slanting upwards far forwards and relatively prominently ridged. Infraorbital foramen normal. Coronoid process usually low. Cheekteeth with upper series characterised in the young by noticeably high cusps; P 3 well developed; the ridges high and the depressions deep, in the main teeth; the small outer third cusp, usually present in Sciurus, is generally absent or scarcely traceable in this group. Lower cheekteeth with four well marked cusps in each tooth, the small subsidiary cusps of Sciurus usually not clear; the central depression tending to be rather smaller than is usual in Sciurus; the transverse ridge extending from the antero-external to anterointernal cusp present.

To these may be added the following characters of the male genital tract. Male genital tract of the NO PENILE DUCT type. Cowper's glands small enclosed in the fibrous capsule of the bulb. In the north Indian pennant's five striped palm squirrel the Cowper's glands are more extensive and the common Cowper's duct extends beyond the bulb to meet the urethra. In others the Cowper's duct joins the urethra in the bulb. Bulbar gland and penile duct absent. Glans penis and os penis well developed. These are species specific in appearance.

Includes only one genus *Funambulus* commonly referred to as palm squirrels. Three groups recognised.

palmarum group: species-*palmarum* (throughout s. India), *pennanti* (five striped throughout N India), *tristriatus* (three buff stripes, Western India and a few areas of south India).

layardi group: Ceylon, includes species *layardi*

sublineatus group: Ceylon and peninsular India, includes species *sublineatus*.

No one, so far has ever been able to define this genus in clear terms. In addition to skeletal characters already too well known the genital tract characters described in this paper as well as in Prasad (1954) may be added. A more comprehensive description of each species will be given in a later publication after a study of the other Ceylonese palm squirrels.

Tamiasciurinae

Range: North America, Canada, Alaska.

Characters:— The generic difference between this and Sciurus is the filiform nature of the glans and the microscopic size of the baculum.

Skull characters much like normal Sciurus; moderately developed postorbital process; braincase not strongly depressed posteriorly. The frontals tend to be slightly depressed. The palate is broad and extending beyond the toothrows. Bullae relatively more enlarged than usual in *Sciurus vulgaris*. Cheekteeth near Sciurus in type. P 3 may be present or absent. In the British Museum specimens they are absent. External characters reminiscent of Sciurus vulgaris type, tail relatively short (40 % of total length), ears tufted seasonally;

sole hairy in most skins seen; also evidently a seasonal character. Digits normal arboreal type.

Example. *Tamiasciurus* with a large number of species and subspecies.

To the above may be added the following characters of the genital tract: The male genital tract is of the NO PENILE DUCT TYPE. The Cowper's glands enclosed in fibrous capsule of the bulb. The common duct opens into the uretha within the bulb. A urethral sinus in the bulb opens into the urethra in the bulb. The penis is long and filiform. The baculum is microscopic. The vagina is highly coiled.

For detailed description of the skull and skeletal characters of Tamiasciurus and its habits and breeding reference may be made to:

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THE DEVELOPMENT OF THE SKULL IN THE SLENDER LORIS, LORIS TARDIGRADUS LYDEKKERIANUS CABR.*

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INTRODUCTION

The adult skull of lemuroids has been the subject of study by a number of workers. FORSYTH-MAJOR (1901) described the orbitotemporal region of lemurs while RAU and SAHASRABUDHE (1930) and HILL (1933, 1936) described the skull of *Loris*. So far, no attempt has been made to study the development of ossifications in a graded series of any lower Primate in order to describe the sequential appearance of the different bones. Similarly a perusal of the literature on the development of the chondrocranium of lemuroids discloses the fewness of investigations. This is largely due to the paucity of suitable embryological material. HENCKEL (1928) who is one of those to study the primordial cranium of lemuroids, showed many points of resemblance with that in the higher Primates and also noted that the early skull of *Tarsius* was very different. It was possible, according to him, to derive the chondrocranium of higher Primates from that of Lemuroidea but not from that of *Tarsius*. I quote from his paper (1928, p. 167):

> Ausserdam ergab sich, dass das Primordialkranium der untersuchten Lemuroiden sich in der gesamtanlage wie in den wichtigsten Einzelmerkmalen dem der höheren Primaten

* The Indian Slender Loris was originally known as *Loris lydekkerianus*. Hill (1933) after a study of the Indian and Ceylonese forms discovered that there were atleast four races of the species and introduced a trinomial nomenclature. I have followed the same.

nähert. Das Primordialkranium von *Tarsius* hingegen zeigt nicht nur keine Annäherung an die höheren Primaten, sondern es weicht unter allen untersuchten Formen der Primatenordnung am meisten von dem gemeinsamen Typus ab. Der Primordialschädel der höheren Primaten lässt sich morphologisch wohl von dem der untersuchten Lemuroiden, aber nicht vom Chondrokranium des *Tarsius* ableiten.

He described the chondrocranium of a 30 mm embryo of *Nycticebus tardigradus* (slow loris). Beyond this, there appears to be no account, as far as I am aware, on the chondrocranium of Lemuroidea. Similarly the chondrocranium of *Tarsius* has been described by FISCHER (1905) and HENCKEL (1927). FRETS (1914) and WEN (1930) have described the nasal region of a number of Primates, including *Tarsius*.

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MATERIAL AND METHODS

I have studied the development of the chondrocranium in the following stages of *Loris* embryos collected by me:

Head-length: 6.5 mm, 8.0 mm, 11.0 mm and 22.0 mm (fully-formed chondrocranium). The development of the centres of ossifications have been followed in the following stages:

Head-length: 8.0 mm, 11.0 mm, 12.0 mm, 15.0 mm, 20.0 mm, 25.0 mm, 27.0 mm, 35.0 mm (postnatal) and the adult skull.

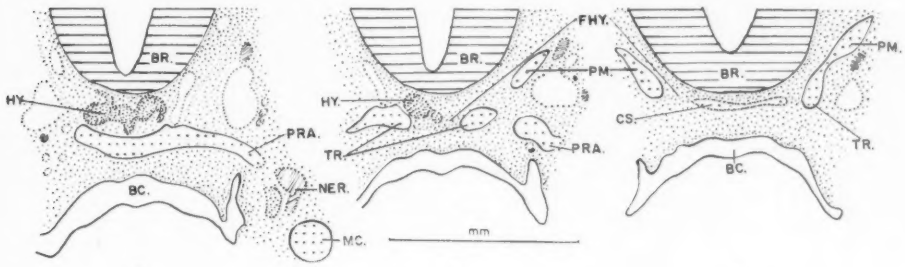
In addition to van Wijhe preparations, sections have been studied with the aid of wax-model reconstructions.

OBSERVATIONS

The Chondrocranium: *Stage 1: 6.5 mm head-length embryo.* Most of the regions of the chondrocranium are noticed as procartilaginous structures.

The occipital region shows the cartilaginous arches prominently. In the posterior region of the basal plate, the occipital arches are united with the former, the hypophysial foramina piercing the occipital arches.

The basal plate is cartilaginous and on either side, the procartilaginous otic capsules are noticed. In the region of the hypophysis, the basal plate continues into a broad cartilaginous plate below it and posteriorly, there is a procartilaginous crista transversa. Figure 1 is in front of the crista and on one side the union of processus alaris (pra) with the side is noticed. Anteriorly to this, there is a broad hypophysial fenestra (Fig. 2, fhy) between the trabeculae. More anteriorly the pilae metopticae (pm) are noticed uniting with the trabecula and at this region, between the two pilae, and ventrally to the brain, the cartila-



Figs. 1, 2, 3. Consecutive transverse sections in the orbitotemporal region of a 6.5 mm (h.—l.) embryo of *Loris*. 1: posterior and 3: anterior.
bc. buccal cavity; br. brain; cs. central stem; fhy. hypophysial fenestra; hy. hypophysis; mc. Meckel's cartilage; ner. nerve; pm. pila metoptica; pra. processus alaris; tr. trabecula.

ginous stem (Fig. 3, cs) is seen as a broad piece of cartilage. Since the exact outline of the stem is not traceable, I have not shown it in the figure.

In the ethmoid region, the nasal septum and paranasals are still procartilaginous. A lamina orbitonasalis is not yet formed.

Of the visceral cartilages, only Meckel's cartilage (Fig. 1, mc) is cartilaginous, the ear ossicles being represented in procartilage.

Stage 2: 8.0 mm head-length embryo: The chondrocranium is more advanced than in the previous stage and most of the regions are cartilaginous.

In the post-auditory region, the two occipital arches are connected by a tectum posterius.

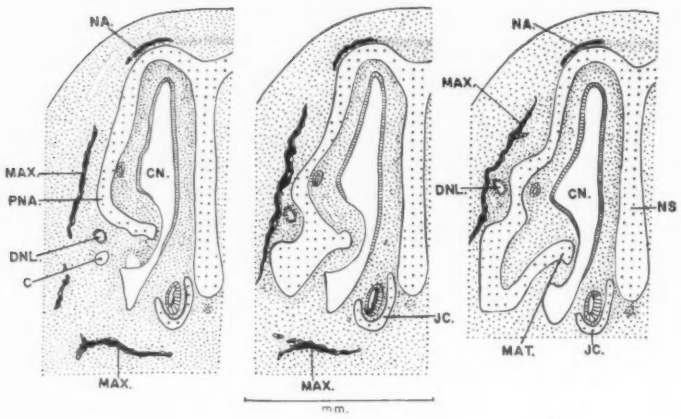
The otic capsules are cartilaginous; the cochlear portion is connected with the basal plate and at this region, the crista transversa is noticed. In the region of the hypophysis, the hypophysial fenestra is obliterated by the growth of cartilage. There is an interesting variation noticed in this region. In front of the optic foramen, a planum supraseptale is formed and slightly posterior to the foramen, the pilae metopticae unite with the central stem. At this region, the processus alaris is noticed and it unites with a broad cartilage below the central stem. This broad cartilage continues into the basal plate while the dorsally situated central stem disappears. This could not have happened by a folding of parts here since other structures are normally disposed.

The lamina orbitonasalis is formed and in the roof, the epiphanial fissure is seen. At the region where the posterior border of the paranasal cartilage joins the parietotectal, a projecting cartilage representing a nasoturbinal is poorly developed. The other turbinals are not yet formed.

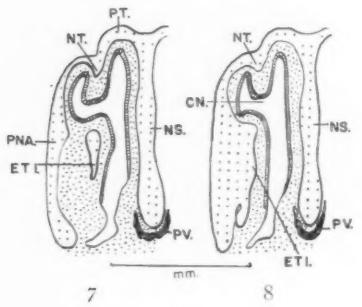
The malleus, incus and stapes are now cartilaginous.

Stage 3: 11.0 mm head-length embryo: The cartilages in the different regions are better developed than in the previous stages. I shall refer to some important points.

There is an interesting variation in the olfactory region. The recessus anterior



Figs. 4, 5, 6. Consecutive transverse sections of the olfactory capsule of a 11 mm (h.—l.) embryo of *Loris*. 4: anterior and 6: posterior.
c. projecting cartilage from the anterior wall of recessus anterior; cn. cavum nasale; dnl. ductus nasolacrimalis; jc. Jacobson's cartilage; mat. maxilloturbinal; max. maxilla; na. nasal; ns. septum nasi; pna. paranasal cartilage.



Figs. 7, 8. Consecutive transverse sections in the lamina orbitonasalis region of a 11 mm (h.—l.) embryo of *Loris*.
cn. cavum nasale; etl. ethmoturbinal; ns. septum nasi; nt. nasoturbinal; pna. paranasal cartilage; pt. parietotectal cartilage; pv. prevomer.

situated laterally to the cavum nasale (Fig. 6, cn) is small and is bounded laterally and ventrally by the paranasal cartilage, the upturned edge of which is the first maxilloturbinal (mat). Anteriorly a broad cartilage forms the wall of the recessus (Fig. 5) and this wall projects further anteriorly as a rounded piece of cartilage (c) whose tip lies ventrally to the nasolacrimal duct (Fig. 4, dnl). This arrangement is rather unusual and the normal condition is described under the fully-formed chondrocranium.

The parietal and supraoccipital cartilages are not chondrified as yet.

The ethmoturbinals have appeared. The first ethmoturbinal appears as a vertical piece of cartilage (Fig. 7, etl) more towards the cavum nasale and posteriorly it unites with the paranasal cartilage (pna) and the latter looks like a thick pillar (Fig. 8, etl) in this region.

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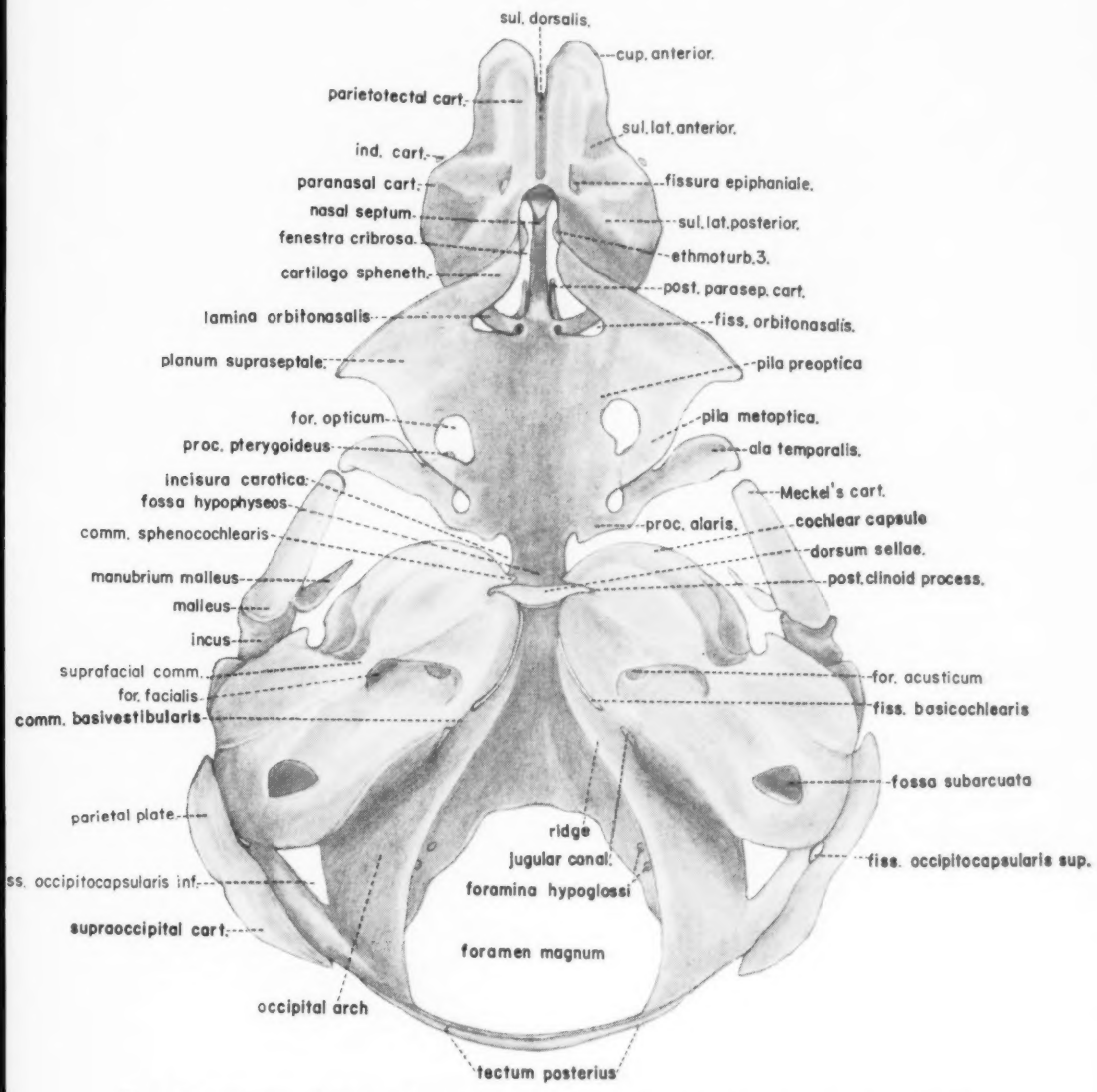

Fig. 9. Dorsal view of the chondrocranium of a 22 mm (h.—l.) embryo of *Loris tardigradus lydekkerianus* Cabr. Meckel's cartilages partly shown. × 50.

Stage 4: 22.0 head-length embryo: The chondrocranium in this stage is completely formed and the basal plate and occipital arches show ossifications.

The occipito-auditory region: Posterior to the auditory capsules, the occipital arches are noticed (Figs. 9—11); ventrally they are united with the basal plate forming the right and left sides to the foramen magnum. Posteriorly to the jugular canal, the otic capsule unites with the occipital arch forming

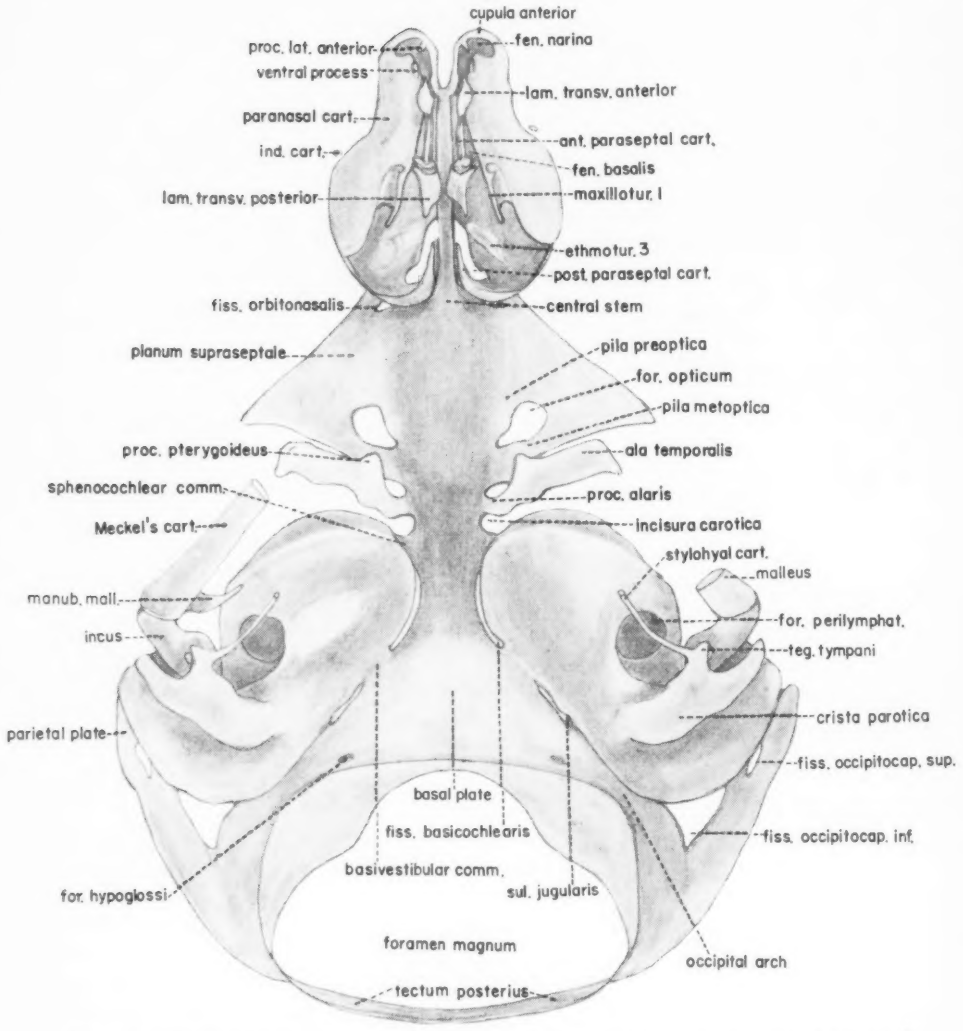

Fig. 10. Ventral view of the chondrocranium of a 22 mm (h.—l.) embryo of *Loris tardigradus lydekkerianus* Cabr. Meckel's cartilages partly shown. ×50.

a deep arch in continuation of the jugular canal and both the canalicular part of the otic capsule and the occipital arch are surmounted by the parietal and supraoccipital cartilages respectively. However, between the occipital arch, supraoccipital cartilage and the posterior edge of each capsule, there are two fissures. The ventral (fissura occipitocapsularis inferior) is larger and looks like the posterior continuation of the jugular canal. The more dorsal smaller (fissura occipitocapsularis superior or jugularis spurius) gives exit to the

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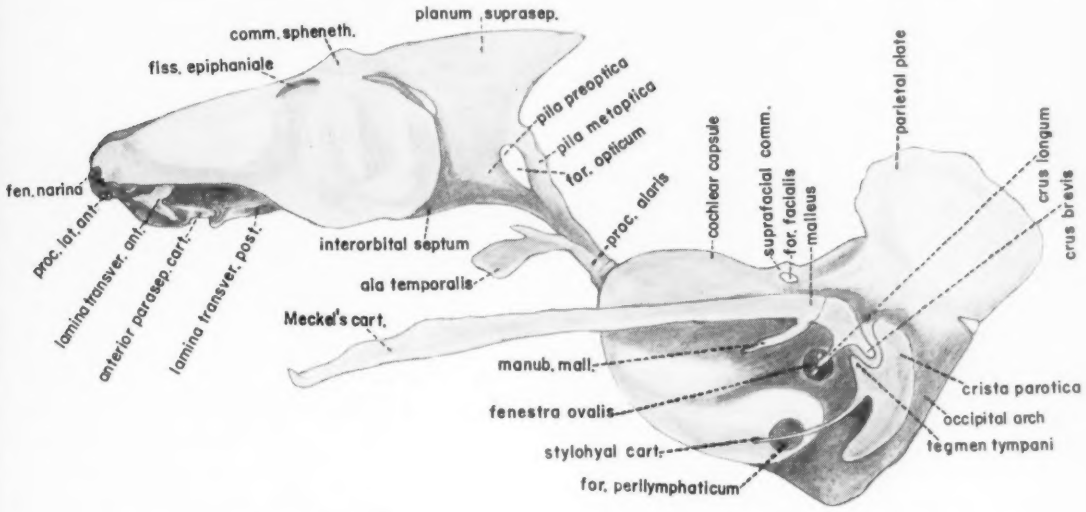

Fig. 11. Side view of the chondrocranium of a 22 mm (h.—l.) embryo of *Loris tardigradus lydekkerianus* Cabr. × 50.

lateral jugular vein. The two supraoccipital cartilages are connected by a cartilaginous tectum posterius.

Mesially each occipital arch shows a prominent ridge (Fig. 9) extending anteroposteriorly, the posterior portion not extending beyond the condylar portion. The foramina hypoglossi, two or three pairs in number, are always situated below this ridge laterally to the condylar portion and are in the form of a canal; a similar canal formation has been noted by HENCKEL (1928) in *Semnopithecus, Chrysothrix* and *Macacus*. FISCHER (1903) also described the variability of the hypoglossal foramina in *Semnopithecus*.

The steep fall in the basal plate from the dorsum sellae towards the foramen magnum in *Loris* is characteristic. This is also noticed in human embryos. In the figures of the chondrocranium of *Semnopithecus* and *Chrysotrix*, I do not notice such a steep incline.

That the auditory capsules exhibit two portions, a canalicular part and a cochlear one is common to all mammals. In *Loris*, the canalicular portion is posterordorsal and is united ventroposteriorly with the occipital arches and is surmounted posterodorsally by the parietal cartilage.

It has been noted by DE BEER (1930) in *Lepus* that the parietal plates and supraoccipital cartilages chondrify separately and then unite with the auditory capsules and occipital arches respectively of either side, the first to unite being the supraoccipital with the occipital arch. In the 11 mm embryo (stage 3) of *Loris*, chondrification of the parietal plate and supraoccipital cartilage has not taken place and being not in possession of stages between this and the 22 mm

(stage 4), I am unable to say if these cartilages appear independently and then fuse. In *Loris,* as in other mammals, the parietal plate appears to continue into the suprafacial commissure. Each auditory capsule is connected with the basal plate also. Anteriorly at the region of the dorsum sellae, the sphenocochlear commissure (Fig. 9) connects the cochlear capsule with the dorsum sellae. Between the jugular canal and the basicochlear fissure is the basivestibular commissure. Posterior to the jugular canal, as already said, the occipitocapsular fissures are noticed. Further, the canalicular part of the capsule gains attachment, laterally to the deep jugular canal with the occipital arch in front of the inferior occipitocapsular fenestra. This is also noticed in the higher Primates (HENCKEL, 1928). The basicochlear fissure is well developed in man and Cercopithecidae (FISCHER, 1903); in *Semnopithecus* and *Macacus* it is absent (HENCKEL, 1928). In *Lemur* and in the platyrrhine *Chrysothrix, Mycetes* and *Hapale,* it is present (HENCKEL, 1928).

On the mesial aspect, the capsule exhibits the acustic foramina (Fig. 9): a larger superior and a smaller inferoposterior. These foramina along with the facial one are noticed in a deep depression. A similar depression is also seen in *Semnopithecus, Macacus* and man (HENCKEL, 1928) but in *Chrysothrix* a depression is absent. In the stage modelled of *Loris,* in addition to the above foramina, facing the smaller orifice, there is a very large gap noticed opening into the cochlear capsule (Figs. 14, 15) which probably is an unchondrified portion of the wall.

Above the auditory apertures, the facial foramen (Fig. 11) and the suprafacial commissure are noticed.

Ventrally, the posterior part of the cochlear capsule shows a circular foramen perilymphaticum (Figs. 10, 11). Through this foramen, one could easily see the large aqueductus cochleae (Fig. 14) which is limited above by a projection from the mesial wall of the cochlear capsule and meets the posterior wall limiting the foramen perilymphaticum. As HENCKEL (1928) described, the foramen perilymphaticum connects on the one hand the brain cavity through the aqueductus cochleae and on the other the capsular cavity. Usually in mammals, there is a cartilaginous projection from the anterior border of the perilymphatic foramen posteriorwards to divide the same into a fenestra rotunda and an aqueductus cochleae. Such a projection is not seen in *Loris.*

Laterally the fenestra ovalis is plugged by the stapes with the stapedial artery passing through it. The crista parotica (Figs. 10, 14) encloses a sulcus facialis and the tegmen tympani is seen dorsally as a prominent projection from the region where the stylohyal gains connexion with the mesial edge of the crista. Ventrally to the root of the styloid process is the lateral opening of the sulcus facialis through which the facial nerve comes out; this later forms the stylomastoid foramen. Mesially to the tegmen, the crus longum (Fig. 14) of the incus and the stapes are accommodated in the fossa incudis; the crus

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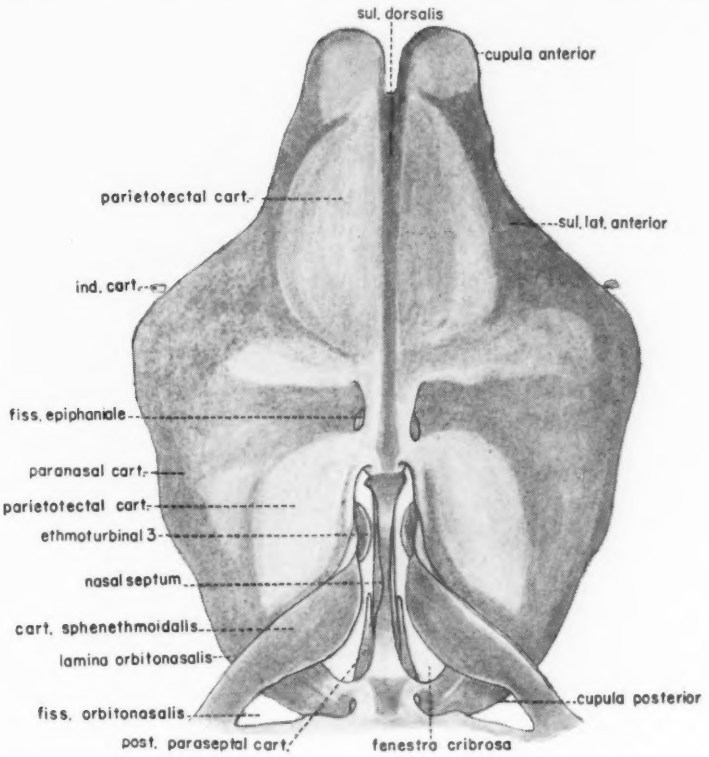

Fig. 12. Dorsal view of the olfactory capsule of a 22 mm (h.—l.) embryo of *Loris tardigradus lydekkerianus* Cabr. × 100.

brevi of the incus is lodged in a fossa in the crista, laterally to the tegmen. The crista parotica (Figs. 10, 11) is a prominent elevation on the ventral aspect of the canalicular part of the capsule (posterior to the tegmen) and this later forms the mastoid portion of the bony skull. In *Tarsius* and *Nycticebus* (HENCKEL, 1928), a crista parotica is also seen; in both a mastoid process is wanting as in *Loris*. A paracondylar process noticed in some lower mammals rabbit, DE BEER, 1930) is also wanting in these Primates.

The suprafacial commissure (Figs. 9, 11, 15) is prominent dorsally on each capsule and it connects the cochlear and canalicular parts and it has already been remarked that it appears as an anterior continuation of the parietal plate. The median part of the tegmen does not send a commissure to fuse with the cochlear capsule or in other words, a lateral prefacial commissure is absent. Mesially, the facial foramen has its secondary opening and laterally to the commissure is the primary orifice. Both the secondary facial and the two acustic foramina are noticed in a deep fossa on the mesial face of the capsule.

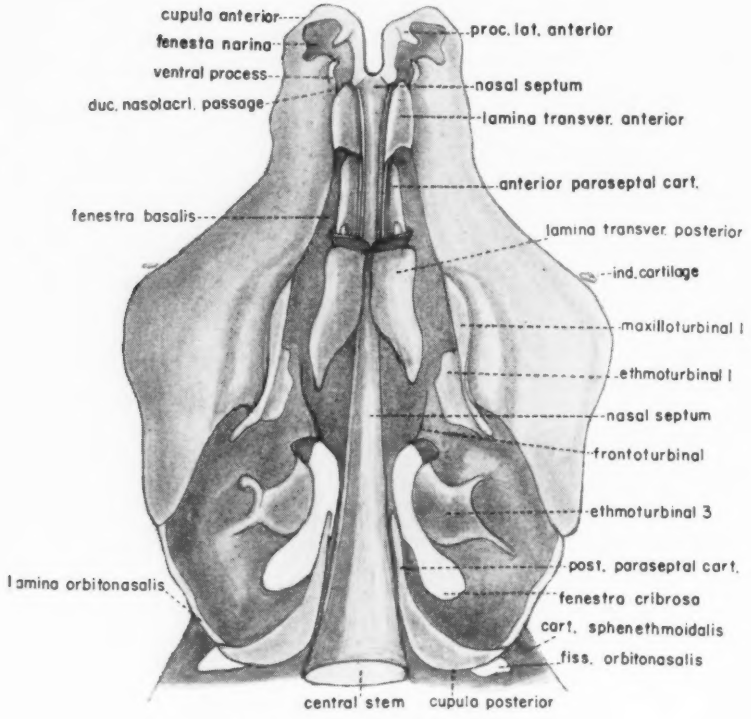

Fig. 13. Ventral view of the same.

In the stage modelled, the suprafacial commissure (Fig. 15) is incomplete; in other stages, it is well developed and complete.

The orbitotemporal region: The orbital cartilages do not extend over the lamina orbitonasalis to a great extent. Here they fuse mesially to the epiphanial fissure forming the cartilago sphenethmoidalis (Figs. 9, 11, 13). A sphenethmoidal commissural cartilage is also noticed in *Nycticebus* (HENCKEL, 1928); in *Tarsius* (HENCKEL, 1927) it is absent. In *Loris* there is an expanded planum supraseptale (Figs. 9, 11) formed by the orbital cartilage in the anterior orbitotemporal region. The orbital cartilage gains attachment with the central stem in front of the optic foramen by a broad preoptic root and the central stem itself forms a fairly broad interorbital septum (however, not visible in the dorsal view). The interorbital septum is very well developed in *Tarsius* according to HENCKEL (1928) and resembles the reptilian condition. Posterior to the optic foramen in *Loris*, there is a narrow metoptic pillar establishing connexion between the stem and the orbital cartilage. The orbital cartilage is *not* connected with the parietal plate posteriorly by an orbitoparietal commissure. In some Primates like *Tarsius*, this commissure is noticed while in

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Nycticebus, Chrysothrix (HENCKEL, 1928) and *Semnopithecus* (FRETS, 1914), it is absent in the stages described.

In front of the dorsum sellae in *Loris,* ventrally from the trabecular region of the central stem cartilage, there arises the cartilaginous basitrabecular process (processus alaris, Figs. 9, 10, 11) which shows at its outer end a broad projection extending horizontally, the ala temporalis, and anteroventrally a pointed one, the pterygoid process. No orifice is noticed in the ala temporalis for the passage of the maxillary branch of the trigeminal. The lamina ascendens in *Chrysothrix, Macacus* and *Homo* shows the foramen rotundum; it is however, absent in *Semnopithecus.* There is no alicochlear commissure in *Loris* and therefore, the carotid foramen is incomplete and it may be called an incisura. An alicochlear commissure is seen in the human embryo described by JACOBY (1898) but absent in the descriptions of other human embryos. It is also absent in *Tarsius, Macacus, Semnopithecus* and *Chrysothrix* (HENCKEL, 1928).

The dorsum sellae (Fig. 9) is met with in the posterior orbitotemporal region in front of the cochlear part of the auditory capsules. Laterally the elevated cartilage of the dorsum sellae unites with the anteromesial wall of the cochlear capsule by the sphenocochlear commissure (Figs. 9, 10), which is in front of the basicochlear fissure. In front of the dorsum sellae is the pituitary fossa in which no orifice is seen as in the early stages of *Lepus.*

The dorsum sellae at its lateral edges gives rise to prominent processes, the posterior clinoid processes (Fig. 9). These do not enclose the abducens nerve or form an incomplete canal as in *Semnopithecus,* the nerve passing in an incisura. In *Tarsius* also, an abducent foramen is not formed. These posterior clinoid processes represent the pilae antoticae of lower amniotes. A dorsum sellae is described in *Macacus, Semnopithecus* (FISCHER, 1903), *Tarsius* (HENCKEL, 1927), *Nycticebus* and *Chrysothrix* (HENCKEL, 1928).

The ethmoid region: The nasal capsule can be studied in three parts. An anterior portion formed by the parietotectal cartilage and projecting in front of the nasal septum, a middle expanded portion formed by the paranasal cartilage and a posterior third region formed by the planum antorbitale or lamina orbitonasalis.

The first and second regions of the capsule are separated by a lateral groove-like sulcus lateralis anterior (Figs. 12, 13) and similarly a shallow sulcus lateralis posterior demarcates the second and third regions. The sulcus dorsalis is the dorsal depression where the parietotectal cartilages have come in contact with the dorsal edges of the nasal septum.

Developmentally, the roof and lateral walls are formed by two independent cartilages. In the middle region, the lateral wall, as already noted, chondrifies as the paranasal cartilage; the roof is formed by the parietotectal cartilage growing from the dorsal edge of the nasal septum. Generally in mammals, the epiphanial foramen marks the region where the fusion between the paranasal

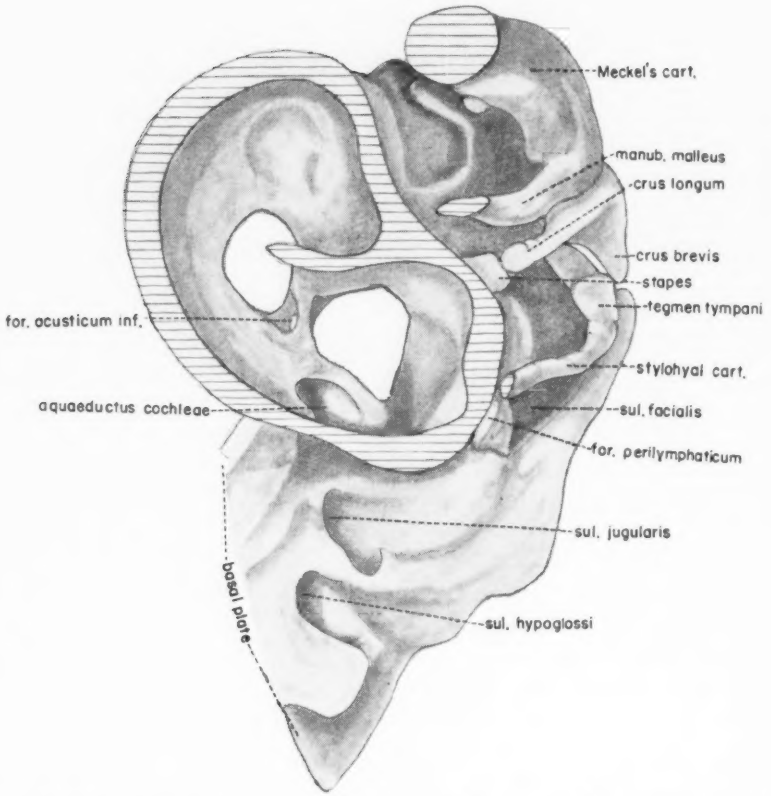

Fig. 14. Anterior view of a part of the otic capsule, basal plate and visceral cartilages of a model of a 22 mm (h.—l.) embryo of *Loris tardigradus lydekkerianus* Cabr. × 100.

and parietotectal has taken place, the opening itself being in the form of a prominent orifice or fissure; in *Loris* the epiphanial fissure is an elongated passage. In the cat, TERRY (1917) recorded a pair of epiphanial openings on either side.

The central stem (Figs. 10, 12, 13) extends from the orbital region anteriorly into the nasal septum. Extending dorsoventrally on either side of the nasal septum is the scroll-like covering of the olfactory sac, which however, is incomplete ventrally both anteriorly and posteriorly to the lamina tranversalis anterior. The gap posterior to the lamina transversalis anterior is the fenestra basalis (Fig. 13) of the nasal capsule. The anterior gap is the fenestra narina which is disposed anterolaterally. The cupula anterior in this region is formed by the parietotectal cartilage; in higher Primates, a front wall for the cupula is absent when the nostrils open directly forwards.

The mesial wall of each cupula is separated from its companion, the common septum separating the two appearing at the antero-mesial end of the lamina

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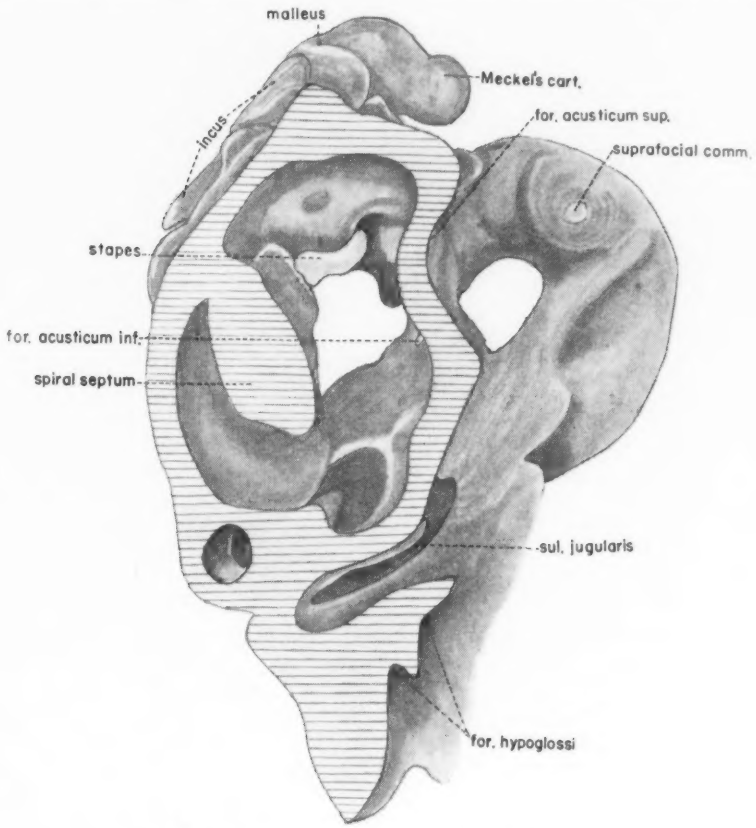

Fig. 15. Posterior view of the same (Fig. 14).

transversalis anterior (Fig. 12). The ventral edge of the mesial wall projects laterally as processus lateralis anterior forming a partial boundary for the anterior naris.

The lower edge of the parietotectal cartilage projects dorsally into the cavity of the cupula and this represents the atrioturbinal (see turbinals). Externally from the ventromesial edge of the parietotectal cartilage, there is a small cartilage projecting anteriorly and I have called this a ventral process (Fig. 10, 13). Posterior to this is the lamina transversalis anterior and between these cartilages, the ductus nasolacrimalis enters the anterior naris (Fig. 5). The passage may therefore be described as being incompletely surrounded by cartilage.

The dorsal cartilaginous projection into the cupula referred to above as atrioturbinal continues posteriorly, separated from the median edge of the parietotectal cartilage and unites with the lateral limb of the lamina transversalis anterior

(or solum nasi). The latter (Figs. 10, 11, 13) is noticed to arise as a rounded piece of cartilage by the side of the projecting anterior end of the nasal septum; this anterior projection of the lamina transversalis anterior may not be comparable to the 'cartilago ductus nasopalatini' of *Lemur* (HERZFELD, 1889) as it is not a ventroanterior projection. The rounded cartilage referred to above assumes posteriorly an U-shape, the lateral limb of which unites with the upturned edge of the parietotectal cartilage. The U-shaped nature of the lamina transversalis anterior is soon lost in sections and the mesial limb of the lamina continues posteriorly by the side of the nasal septum as the anterior paraseptal cartilage. As the lamina transversalis anterior does not unite with the nasal septum, a complete zona annularis is absent in *Loris*. Peculiarly, in *Macacus* (FRETS, 1914) and man, a lamina transversalis anterior is not formed; in *Nycticebus* (HENCKEL, 1928) the zona is incomplete while a zona annularis is noticed in *Tarsius* (HENCKEL, 1927), *Propithecus* and *Lemur* (FRETS, 1914).

The anterior paraseptal cartilage continues posteriorly into the U-shaped lamina transversalis posterior or Jacobson's cartilage in *Loris*. The two laminae may approximate ventrally to the nasal septum (Fig. 13). Posteriorly, the laminae end blindly.

Posterolaterally to the sulcus lateralis anterior and externally to the paranasal cartilage on either side of the olfactory capsule, a small independent piece of cartilage (Figs. 9, 10, 12, 13, ind. cart) is noticed. I am unable to say what the exact significance of this cartilage is.

The third region of the olfactory capsule in *Loris* is the planum antorbitale or lamina orbitonasalis, the roof of which is formed by the tectum nasi posterius (parietotectal cartilage) and the floor by a short piece of cartilage, the posterior paraseptal cartilage; the posterior cupshaped portion is the cupula posterior (Figs. 10, 13). The mesial wall of the cupula is free from the septum in the majority of mammals but it may establish secondary connexions with it (*Sorex, Lepus*); this is also the case in *Loris*. The nasal capsule and the orbital cartilages are connected by the well developed cartilago sphenethmoidalis (Figs. 9, 11, 13) laterally to the foramen advehentia and mesially by the orbital cartilages uniting with the nasal tectum between two small orifices in the latter. The lamina orbitonasalis does not project far into the orbital region so that the ala orbitalis or orbital cartilage just rests on the posterior wall of the cupula and ventrally, only a small portion of the central stem is visible as the interorbital septum in this region (Figs. 10, 13). Also in *Tarsius*, the orbital cartilages do not cover the cupula posterior whose posterior wall is incomplete according to HENCKEL (1928).

The floor of the capsule in the posterior region is formed by the lamina orbitonasalis and posterior paraseptal cartilage as noted above. While in *Lepus* (DE BEER, 1930) the paraseptal cartilages being continuous (cartilago paraseptalis) forms an uninterrupted floor by the side of the nasal septum, in *Loris*,

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there is a wide gap between the two. In *Tarsius* FRETS' (1914) description appears to indicate an uninterrupted paraseptal cartilage. In *Nycticebus* the paraseptal cartilages are however, incomplete. Except for the secondary connexion mentioned above between the septum and the medial wall of the lamina orbitonasalis in *Loris,* the olfactory capsule is independent of the median septum. The secondary connexion is obviously for anchoring the capsule in this region.

In the model (Figs. 9, 11) the lamina cribrosa riddled with a number of orifices for the passage of the ramuli of the olfactory nerves into the olfactory sacs is taken off and the dorsal edge of the septum nasi, slightly depressed in the middle, is noticed. The limitation of the fenestra cribrosa may now be defined as follows: the anterior border of the tectum nasi posterius posteriorly, the upper edges of the lamina orbitonasalis laterally and anteriorly the roof of the nasal capsule. The septum nasi would form the common median boundary for both fenestrae cribrosae. Peculiarly in *Tarsius* (HENCKEL, 1927), there is no lamina cribrosa. According to HENCKEL (1928), a crista galli is absent in the lower Primates examined by him.

Generally in the mammals, there is a large fissure between the orbital cartilages and the posterior part of the lamina orbitonasalis, the fissura orbitonasalis. In *Loris* as the orbital cartilage is as broad as in *Nycticebus* (HENCKEL, 1928), there is in front of the preoptic root, a small orbitonasal fissure (Figs. 9, 10, 12, 13) looking over the tectum posterius of the lamina orbitonasalis.

A foramen dorsale is absent and the ramulus lateralis nasi of the ethmoid branch of the trigeminal nerve runs dorsally to the cribriform plate.

The turbinals: A number of projections from the wall of the nasal capsule cuts the internal space. It has already been stated that the side wall in front of the lamina orbitonasalis of the nasal capsule arises from the paranasal cartilage while the roof is formed by the parietotectal cartilage.

The atrioturbinal, as already pointed out, is the anteriormost of the turbinals. In *Loris* it is a dorsal projection from the median edge of the parietotectal cartilage of the cupula anterior. Tracing the sections posteriorly, it is noticed that the atrioturbinal remains a separate piece of cartilage from the paranasal edge in a few sections, and posterior to the nasolacrimal duct, it unites with the lamina transversalis anterior cartilage. As has been described by DE BEER (1929) in *Sorex,* the atrioturbinal may be considered as the anterior part of the maxilloturbinal. I do not find a reference to this turbinal cartilage in the prosimiae studied by FRETS (1914).

The first maxilloturbinal appears as a short projection of the paranasal cartilage into the olfactory cavum. This continues posteriorly (Figs. 10, 13) until it separates itself from the paries nasi and is noticed in a few sections as an independent piece of cartilage. In *Propithecus* (FRETS, 1914) the first maxilloturbinal is in the form of a footplate. In *Loris,* a downward projection

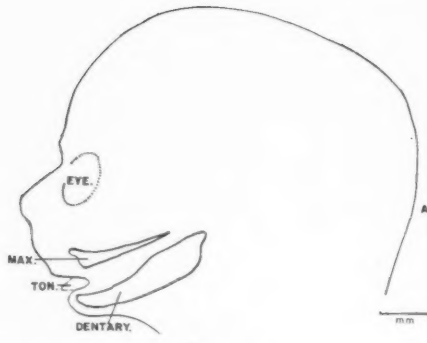

Fig. 16.

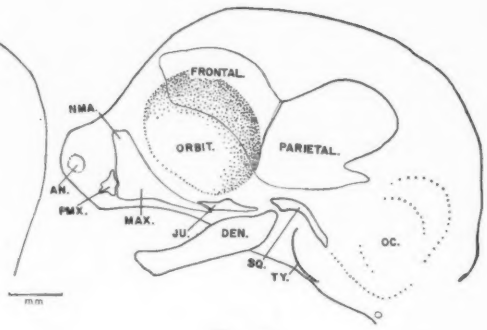

Fig. 17.

Fig. 16. Side view of the alizarin transparency of the head of a 8 mm (h.—l.) embryo of *Loris*.
max. maxilla; ton. tongue.

Fig. 17. Side view of the alizarin transparency of the head of a 11 mm (h.—l.) embryo of *Loris*.
an. anterior naris; den. dentary; ju. jugal; max. maxilla; nma. nasal process of maxilla; oc. otic capsule; pmx. premaxilla; sq. squamosal; ty. tympanic.

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from the parietotectal cartilage appears where the first maxilloturbinal forms along with the ventral wall, a floor for the recessus supraconchalis (rec. maxillaris according to FRETS). FRETS (op. cit.) shows an exactly similar downward projection in *Propithecus* (his figs. 19, 20) and calls it processus uncinatus and another projection also from the roof (at the root of the uncinate process) is called the nasoturbinal. The nasal cavity laterally to the uncinate process is called rec. maxillaris by him. If the uncinate process united with the lateral cartillage, it would enclose the recessus maxillaris (into which glands are noticed to open) and would resemble Figure 12 drawn by DE BEER (1929) for the shrew. The cartilage projecting downward in front of the crista semicircularis is called by him 'nasotrubinal' in the shrew. Similary in the cat TERRY (1917) shows the anterior portion as the nasoturbinal, while posteriorly, it is the crista semicircularis. In *Loris* also, the downwardly projecting cartilage or nasoturbinal (Text-figs. 7, 8, nt) does not posteriorly unite with the paranasal to form a crista semicircularis. While in *Propithecus* (FRETS, 1914) and *Loris*, the recessus maxillaris is incompletely bound by cartilage, in *Tarsius*, it is enclosed on all sides.

The first ethmoturbinal (Fig. 13) appears in *Loris* as a small circular piece of cartilage in the olfactory sac at about the middle region of the nasoturbinal or uncinate process of FRETS. Posteriorly to where the anterior root of the first ethmoturbinal is noticed in the sidewall, the olfactory sac is noticed as a recessus frontalis above and a recessus maxillaris below; the latter is a continuation of the rec. maxillaris already referred to. In the shrew (DE BEER, 1929) the crista semicircularis appears anterior to the first ethmoturbinal and the median olfactory sac is separated from the lateral rec. anterior. I have already

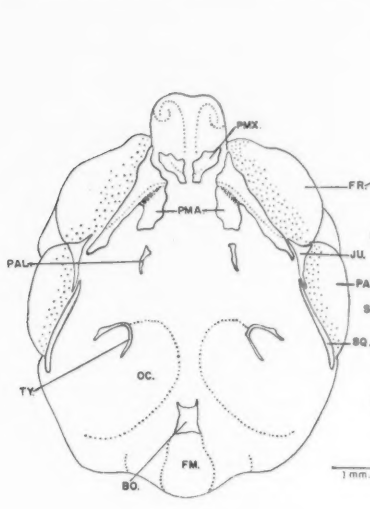

Fig. 18.

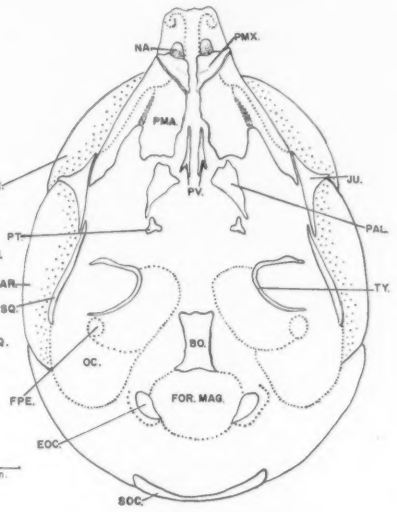

Fig. 19.

Fig. 18. Ventral view of the alizarin transparency of the head of a 11 mm embryo of *Loris*. bo. basioccipital; fm. foramen magnum; fr. frontal; ju. jugal; oc. otic capsule; pal. palatine; par. parietal; pma. palatinal process of maxilla; pmx. premaxilla; sq. squamosal; ty. tympanic.

Fig. 19. Ventral view of the alizarin transparency of the head of a 12 mm (h.—l.) embryo of *Loris*.
bo. basioccipital; eoc. exoccipital; fpe. foramen perilymphaticum; ju. jugal; na. nasal; oc. otic capsule; pal. palatine; par. parietal; pma. palatine process of maxilla; pmx. premaxilla; pt. pterygoid; pv. prevomer; sq. squamosal; ty. tympanic.

stated that in *Loris* a crista semicircularis is absent unless it be that the downwardly projecting cartilage represents a conjoint one. In the shrew (DE BEER, 1929) the nasoturbinal fuses posteriorly with the cartilago semicircularis.

Posterior to the uncinate process, the dorsal edge of the paranasal cartilage projects mesioventrally as the frontoturbinal (Fig. 13). In the same region, the second ethmoturbinal appears (not shown in my Figures) as a rounded piece of cartilage posterior to the first, and unites with the side wall posteriorly; the second ethmoturbinal is smaller than the first. The third ethmoturbinal (Figs. 9, 10, 12, 13) is visible through the fenestra cribrosa; in a dorsal view it appears as a piece of rounded cartilage by the side of the lamina orbitonasalis. It becomes elongated posteriorly and establishes contact with a projecting cartilage from the side wall above. The root of the third ethmoturbinal is situated posteriorly.

The osteocranium: With a view to examine the centres of ossifications in *Loris*, several stages of the embryos were stained with alizarin and studied. I shall briefly describe the ossifications in the various stages.

Stage A: 8.0 mm head-length embryo: This is the earliest stage examined

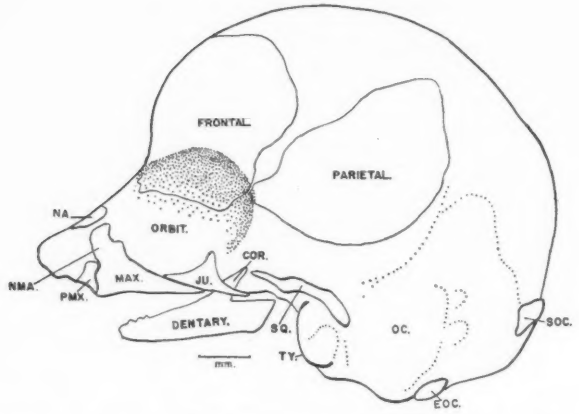

Fig. 20. Side view of the alizarin transparency of the head of a 12 mm (h.—l.) embryo of *Loris*.
cor. coronoid process; eoc. exoccipital; ju. jugal; max. maxilla; na. nasal; nma. nasal process of maxilla; oc. otic capsule; pmx. premaxilla; soc. supraoccipital; sq. squamosal; ty. tympanic.

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and only two ossifications have manifested themselves, the maxillae (Fig. 16, max) and the dentaries. While the maxillae show tiny nasal processes, the dentaries are free of their characteristic processes.

Stage B: 11.0 mm head-length embryo: Additional bones have appeared. The frontal (Fig. 17, 18, fr) is noticed partly in the orbital region and partly in the supraorbital region: the parietal (par) closely follows the posterior boundary of the frontal and there is no orbital wing. Both the bones have not yet extended dorsally.

The nasal process of the maxilla (nma) is prominent and posteriorly the maxilla is in contact with the triradiate jugal (ju) or malar; mesially the palatal plate (Fig. 18, pma) of the maxilla has appeared. In front of the maxillae, the premaxillae (pmx) are noticed. The squamosal (sq) is noticed as an arch in front of the auditory capsule. A bow-shaped tympanic (ty) has appeared.

On the palatal aspect, posterior to the palatal plate of the maxilla (pma), the palatal part of the palatine (pal) is seen, with the posterior portion pointed.

Anterior to the foramen magnum, in the basal plate the basioccipital (Fig. 18, bo) has appeared, both dorsally and ventrally of the plate. This is the only cartilage bone at this stage and appears as a single centre of ossification.

The mandible (Fig. 22) shows a small coronoid and a condylar prominence.

Stage C: 12.0 mm head-length embryo: Certain advancement has been made in the ossifications of this stage over the previous.

The frontals and parietals (Fig. 20) are noticed to extend in the roof. The nasals (na) have appeared. Ventrally paired prevomers (Fig. 19, pv)

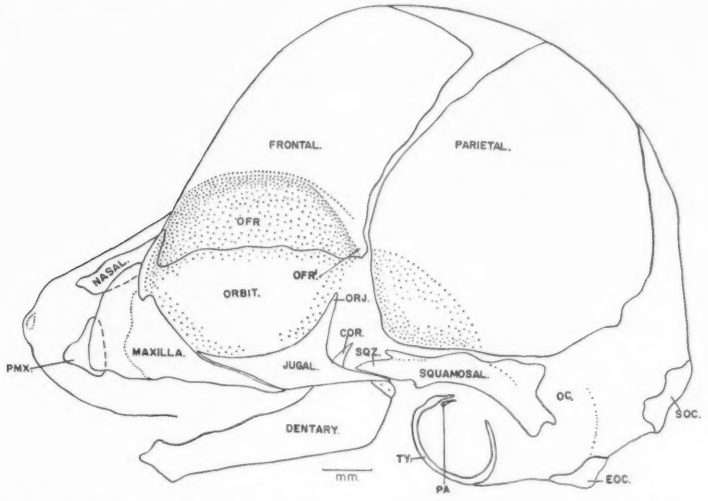

Fig. 21. Side view of the alizarin transparency of the head of a 15 mm (h.—l.) embryo of *Loris*.
cor. coronoid process; eoc. exoccipital; oc. otic capsule; ofr. orbital part of frontal; ofr[1]. postorbital process of frontal; orj. orbital process of jugal; pa. prearticular; pmx. premaxilla; soc. supraoccipital; sqz. zygoma of the squamosal; ty. tympanic.

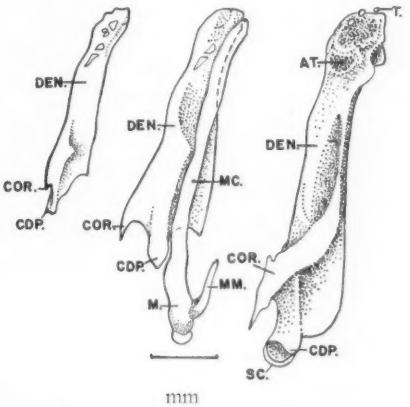

Fig. 22. Dorsal view of the left ramus of the lower jaw of *Loris* embryos 11 mm, 12 mm and 15 mm respectively; in one Meckel's cartilage is shown.
at. tooth alveolus; cdp. condylar process; cor. coronoid process; den dentary; m. malleus; mc. Meckel's cartilage; mm. manubrium malleus; sc. secondary cartilage; t. tooth.

are noticed between the palatal plates of maxillae (pma) which latter have incraesed considerably in size. Posterior to the palatines, the pterygoids (pt) are noticed as triangular ossifications and are not connected with the processus alaris.

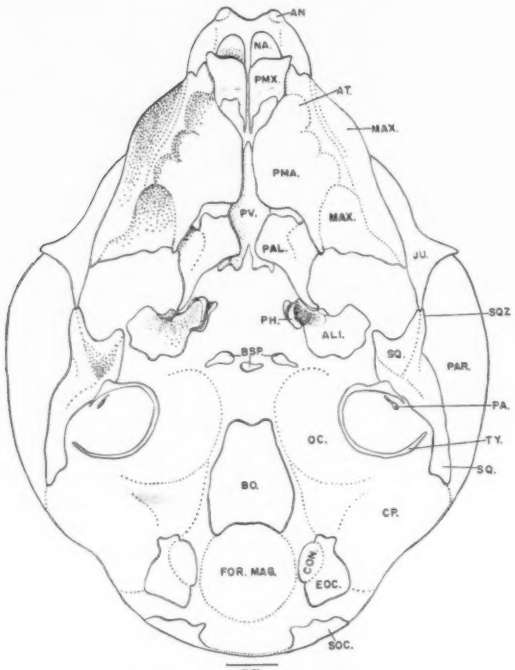

Fig. 23. Ventral view of the alizarin transparency of the head of a 15 mm (h.—l.) embryo of *Loris*.

ali. alisphenoid; an. anterior naris; at. tooth alveolus; bo. basioccipital; bsp. basisphenoid; con. condyle; cp. crista parotica; eoc. exoccipital; ju. jugal; max. maxilla; na. nasal; oc. otic capsule; pa. prearticular; par. parietal; ph. pterygoid hamulus; pma. palatal process of maxilla; pmx. premaxilla; pv. prevomer; soc. supraoccipital; sq. squamosal; sqz. zygomatic process of squamosal; ty. tympanic.

The zygomatic process of the squamosal has not established connexion with the malar or jugal (Fig. 20, ju).

The exoccipitals (in the condylar portion) (eoc) and the supraoccipital (soc) (two broad lateral portions connected by a bridge) have appeared.

The mandible shows a prominent coronoid process (Figs. 20, 22, cor) posteriorly and a few alveoli anteriorly. The condylar process (Fig. 22, cdp) has not developed secondary cartilage in the 11 and 12 mm stages.

Stage D: 15.0 mm head-length embryo: The frontals (Fig. 21) and parietals form roofing bones dorsally. The frontal is noticed to send a small postorbital limb (ofr[1]) towards the jugal process (orj), which later forms a postorbital bar. The frontal process of the jugal (orj) is prominent.

The nasal process of the maxilla overlaps the nasal and similarly the premaxilla (pmx) is overlapped by the maxillary process. These two bones show ventrally thecae (Fig. 23, at) for teeth.

The two centres of the prevomer (pv) have united anteriorly and this

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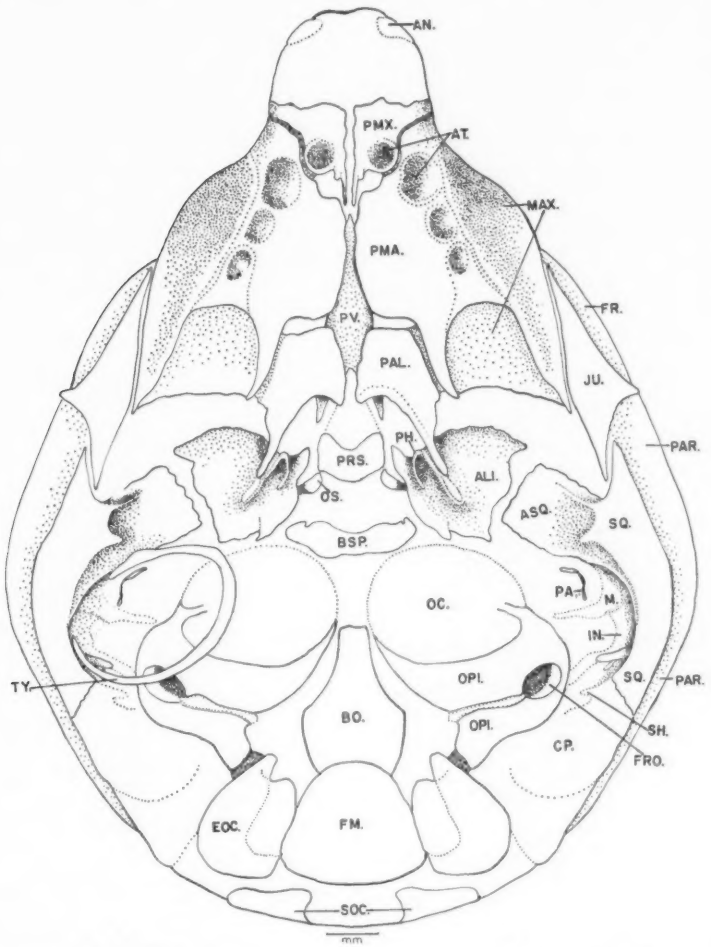

Fig. 24. Ventral view of the alizarin transparency of the head of a 20 mm (h.—l.) embryo of *Loris*.

ali. alisphenoid; an. anterior naris; asq. alisphenoid limb of squamosal; at. alveoli for teeth; bo. basioccipital; bsp. basisphenoid; cp. crista parotica; eoc. exoccipital; fm. foramen magnum; fr. frontal; fro. fenestra rotunda; in. incus; ju. jugal; m. malleus; max. maxilla; oc. otic capsule; opi. opisthotic; os. orbitosphenoid; pa. prearticular; pal. palatine; par. parietal; ph. pterygoid hamulus; pma. palatinal process of maxilla; pmx. premaxilla; prs. presphenoid; pv. prevomer; sh. stylohyal cartilage; soc. supraoccipital; sq. squamosal; ty. tympanic.

portion is partially enclosed by the extending palatal plates of maxilla (pma). Posteriorly the palatal portion of palatines (pal) are also noticed to enclose the prevomer which shows the dual nature here.

The posterior limb of the jugal is contacted by the zygomatic process (Fig.

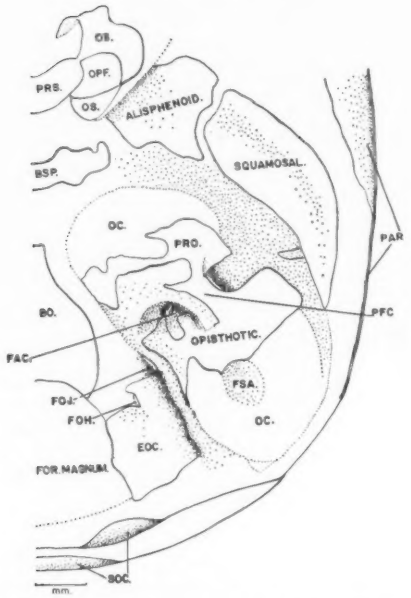

Fig. 25. Dorsal view of the right half of the alizarin transparency of the head of a 20 mm (h.—l.) embryo of *Loris*, roof taken off.
bo. basioccipital; bsp. basisphenoid; eoc. exoccipital; fac. acustic foramina; foh. condylar foramen; foj. foramen lacerum posterius; fsa. fossa subarcuata; oc. otic capsule; opf. optic foramen; os. orbitosphenoid; par. parietal; pfc. prefacial commissure; pro. prootic; prs. presphenoid; soc. supraoccipital.

21, sqz) of the squamosal forming a zygoma. The squamosal also sends a limb towards the otic capsule (Fig. 23).

Dorsally, in the metoptic root, a small orbitosphenoid ossification can be seen.

The limb of the tympanic (ty) towards the squamosal is bladelike and posteriorly to it, there is a splintlike bone, the prearticular (pa).

A platelike ossification is noticed to be continuous with the pterygoid nodule seen in the previous stage. While the posterior part is an ossification in the basipterygoid process and therefore, represents the alisphenoid (Fig. 23, ali), the anterior portion is probably membranous where the processus alaris does not extend. The pterygoid hamulus (ph) includes a fossa laterally.

The basisphenoid (bsp) is noticed to ossify from three separate centres, two lateral and a smaller median.

In the dentary, the condylar process has a cartilaginous epiphysis (Fig. 22, sc).

The occipitals (bo, eoc, soc) have increased in size; the exoccipitals show prominent condyles (con).

Stage E: 20.0 mm head-length embryo: Certain important changes are noticed

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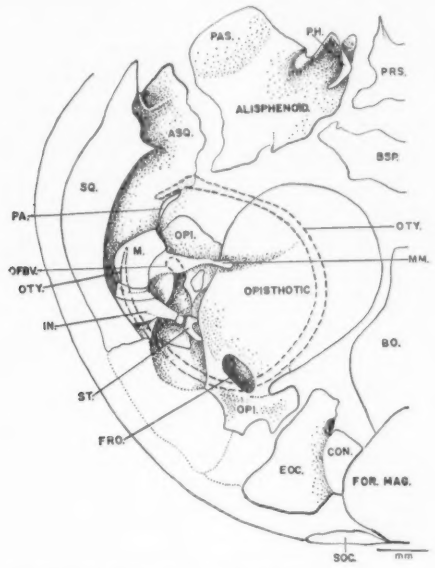

Fig. 26. Ventral view of the left half of an alizarin transparency of the head of a 25 mm (h.—l.) embryo of *Loris*.
asq. alisphenoid limb of squamosal; bo. basioccipital; bsp. basisphenoid; con. condyle; eoc. exoccipital; fro. fenestra rotunda; m. malleus; mm. manubrium malleus; ofbv. outline of a foramen for a blood vessel; opi. opisthotic; oty. outline of tympanic; pas. parasphenoid portion of alisphenoid; ph. pterygoid hamulus; prs. presphenoid; soc. supraoccipital; sq. squamosal; st. stapes.

in the palatal aspect of this embryo. In the orbital region, a complete postorbital bar is not yet formed.

The palatine (Fig. 24, pal) has sent a prominent process laterally to the pterygoid humulus (ph). Between the two alisphenoids (ali), the presphenoid (prs) has appeared as a broad median centre of ossification. Posterolaterally to the presphenoid, there are two smaller rounded ossifications. The latter are ossifications in the metoptic roots and they are the orbitosphenoids (os) already seen in the previous stage. On one side, there is already indication of the orbitosphenoid uniting with the presphenoid.

In front of the dorsum sellae, the basisphenoid (bsp) is noticed as a single broad ossification. A considerable gap exists between the basisphenoid and presphenoid ossifications. The squamosal has a prominent broad limb (asq) towards the alisphenoid (ali).

The auditory capsules show ossification. On the ventral aspect, the posterior portion of the cochlear capsule (oc) in front of the fenestra rotunda and also posteriorly to it extending to the exoccipital (eoc) are ossified; this ossification extends dorsally in front of the fossa subarcuata (Fig. 25, fsa) and posteriorly to the foramen acusticum (fac). This represents the opisthotic (opi). Dorsally

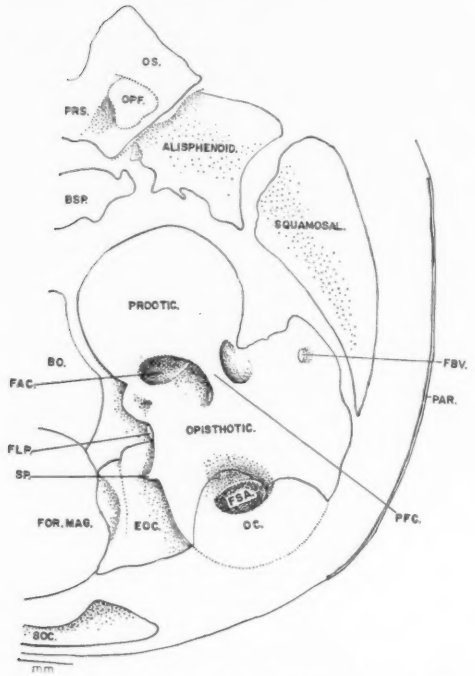

Fig. 27. Dorsal view of the right half of the interior of the head of a 25 mm (h.—l.) embryo *of Loris.*
bo. basioccipital; bsp. basisphenoid; eoc exoccipital; fac. acustic foramen; fbv. foramen for blood vessel; flp. foramen lacerum posterius; fsa. fossa subarcuata; oc. otic capsule; opf. optic foramen; os. orbitosphenoid; par. parietal; pfc. prefacial commissure; prs. presphenoid; sp. spinous process.

in front of the foramen acusticum, the otic capsule shows also another ossification (pro) which is continuous with the opisthotic at the prefacial commissure (pfc). This anterior ossification represents the prootic (pro).

Of the visceral arches, stapes and malleus (Fig. 24, mal) are unossified and a part of the incus (in) is ossified.

Stage F: 25.0 mm head-length embryo: Considerable increase in the size of bones has taken place.

The postorbital bar formed by frontal and jugal is still incomplete.

The presphenoid (Figs. 26, 27, prs), alisphenoid and basisphenoid (bsp) are still independent of each other; however, the orbitosphenoids (os) have united with the presphenoid already noticed in the previous stage. The alisphenoid has not yet established contact with the mesial limb of the squamosal (Fig. 26, asq).

The otic capsules show extensive ossification. The opisthotic has extended considerably in front of the fenestra rotunda (fro) over the otic capsule and dorsally the prootic (Fig. 27, pro) and opisthotic have fused so that no demar-

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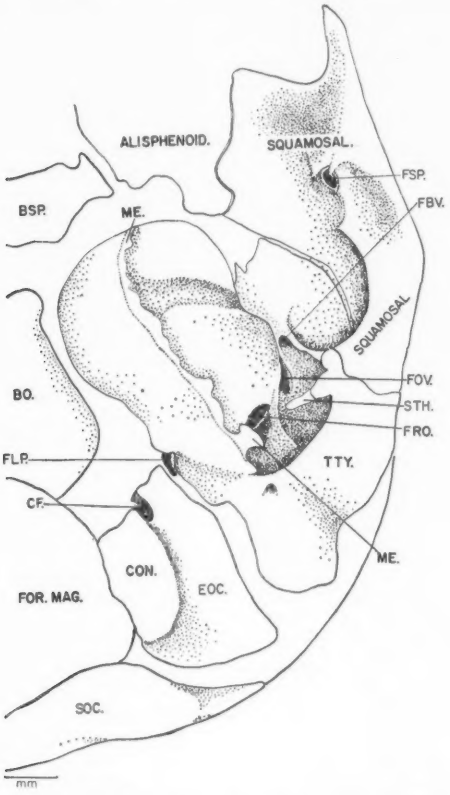

Fig. 28. Ventral view of the right half of the alizarin transparency of the head of a 27 mm (h.—l.) embryo of *Loris*, auditory region.
bo. basioccipital; bsp. basisphenoid; cf. condylar foramen; con. condyle; eoc. exoccipital; flp. foramen lacerum posterius; fov. foramen ovalis; fro. fenestra rotunda; fsp. foramen spinosum; me. mastoid extension; soc. supraoccipital; sth. stylohyal; tty. tegmen tympani.

cation is possible. The opisthotic appears to extend further laterally; it extends dorsally to the sulcus facialis and meets the squamosal extension (Fig. 26, asq). Posterior to the fossa subarcuata (Fig. 27, fsa) the otic capsule (oc) is still cartilaginous. The tympanic (Fig. 26, oty) has grown in size and is free.

In the ear ossicles, the posterior end of the prearticular (pa) has fused with the processus anterior of the malleus (m), which latter is completely ossified. The incus (in) and stapes (st) are also bony.

Stage G: 27.0 mm head-length embryo: The postorbital bar is still incomplete. The lateral extension of the alisphenoid (Figs. 28, 29) has met the medial extension of the squamosal. The basisphenoid (bsp), alisphenoid and presphenoid (prs) are separate.

Except for a short portion posterior to the fossa subarcuata (Fig. 29, fsa)

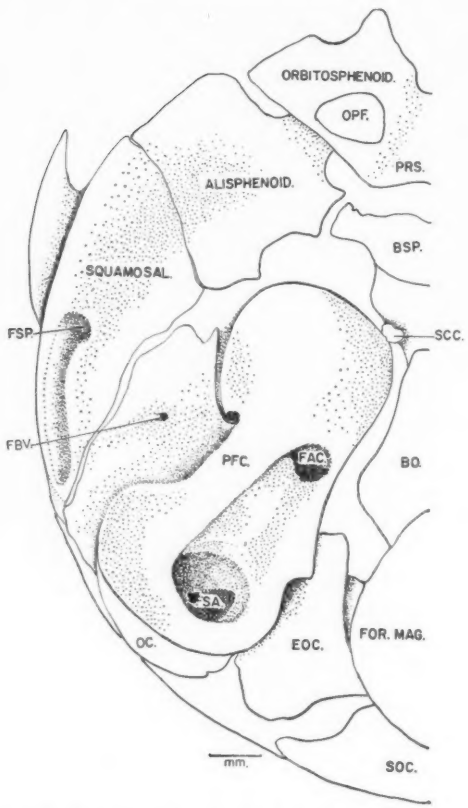

Fig. 29. Dorsal view of the left half of the interior of the head of a 27 mm (h.—l.) embryo of *Loris*.
bo. basioccipital; bsp. basisphenoid; eoc. exoccipital; fac. acustic foramen; fbv. foramen for a blood vessel; fsa. fossa subarcuata; fsp. foramen spinosum; oc. otic capsule; opf. optic foramen; pfc. prefacial commissure; prs. presphenoid; scc. sphenocochlear commissure; soc. supraoccipital.

dorsally, complete ossification of the otic capsule has taken place. From the median ventral wall of the otic capsule, mastoid extension (Fig. 28, me) has just commenced and therefore, the fenestrae rotunda (fro) and ovalis (fov) are still visible in the ventral aspect of the skull. The ossified tegmen tympani (tty) is not yet united with the squamosal. The tympanic bone is also free.

Stage H: 35.0 mm head-length embryo: This stage represents a postnatal juvenile one.

In the auditory region, the tympanic (Fig. 30, ty) has increased in size and has fused with the periotic, but the outline of it can be easily made out; the squamosal has fused with this giving rise to the temporal bone (sqt). Lateral extension of the tympanic to enclose the external auditory meatus (eam) has not taken place somuch so the tympanic membrane (removed to show the

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ossicles in Fig. 30) is visible. The mastoid has grown enclosing air spaces and the fenestrae ovalis and rotunda are no more visible externally.

The basisphenoid is united on either side anterolaterally with the alisphenoid. Posteriorly the basisphenoid is separate from the basioccipital. Anteriorly the presphenoid is also separate; the gaps between these ossifications are considerably bridged.

The nasal region shows certain advancements. While in the 27 mm stage (stage G), the turbinals were still cartilaginous, in this stage, they are all ossified. The nasal septum is ossified into a median bony mesethmoid plate. On either side of it, the ethmoturbinals are noticed. The maxilloturbinal has not assumed the final shape of a scroll but its fusion with the maxilla has taken place. The nasals are free from turbinals as also in the adult. However, RAU and SAHASRABUDHE (1930, p. 186) noted that 'The nasotribunal which begins at the level of the last premolar, extends nearly upto the level of the canine'. Obviously they were referring to a part of the ethmoid, usually described as a nasoturbinal.

The lateral portion of the bifid prevomer has united with the posterior portion of the ethmoturbinal.

The occipital bones are still separate.

The lower jaw shows the prominent coronoid and condylar processes. The condyle is an ossification in the secondary cartilage. The appearance of this secondary cartilage as an epiphysis was noticed in the 15 mm stage. The milk dentition is being replaced; the molars are lodged in deep sockets.

I shall now tabulate the appearance of the different centres of ossifications and also the fusion of some of them in the stages of *Loris* studied by me. Comparison with other Primates is not possible for a similar study has not been made, as far as I am aware, in any other Primate except man.

Stages:	Centres of ossification:	Fusion of Centres:
8 mm	Dentary, Maxilla	—
11 mm	Premaxilla, Squamosal, Tympanic, Frontal, Parietal, Jugal, Palatine, Basioccipital.	—
12 mm	Nasal, paired Prevomers, Pterygoid, Supraoccipital, Exoccipital.	—
15 mm	Prearticular, Orbitosphenoid, alisphenoid, Basisphenoid (three centres).	Prevomers fuse to form a median ossification; Pterygoid fuses with alisphenoid.
20 mm	Presphenoid, Opisthotic, Prootic.	Basisphenoid centres fuse; Opisthotic and prootic are fused.
25 mm	Malleus, Incus, Stapes.	Orbitosphenoid fuses with Presphenoid; Prearticular fuses with malleus.
35 mm	Turbinals, Mesethmoid.	Periotic fuses with tympanic and squamosal; basisphenoid fuses with alisphenoid.

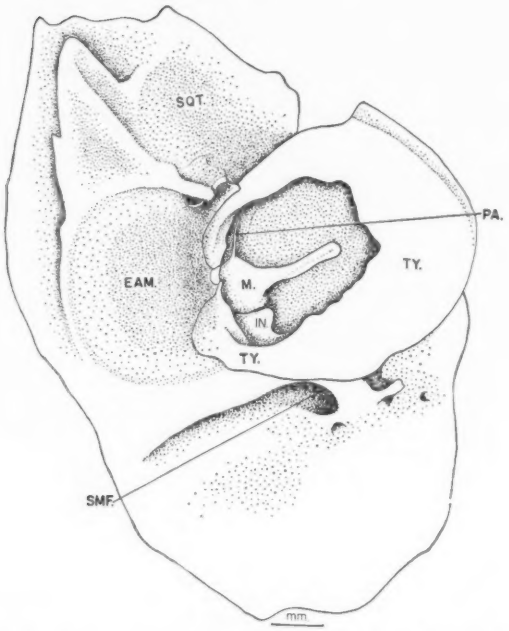

Fig. 30. Ventral view of the alizarin transparency of the left temporal bone of a 35 mm (h.—l.) embryo of *Loris*.
eam. external auditory meatus; in. incus; m. malleus; pa. prearticular united with malleus; smf. stylomastoid foramen; sqt. squamous-temporal bone; ty. tympanic still growing.

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The adult skull: The adult skull of *Loris* has been described by RAU and SAHASRABUDHE (1930) and later by HILL (1933, 1936). I shall however, refer to only a few important aspects of the skull in view of the developmental study I have made.

In the nasal region, the prevomer becomes closely applied to the ventral portion of the mesethmoid and it was noticed above, that the posterior portion of the prevomer united with the ethmoturbinal. I do not know if this union represents an ossification in the lamina orbitonasalis. Anteriorly, the outline of the prevomer can be made out in the alizarin transparencies but it is firmly attached to the mesethmoid. The united ethmoid and prevomer can be seen posterior to the palatal plate of palatine in the dry skull. In describing the orbital region of lemurs and monkeys, FORSYTH-MAJOR (1901, p. 140) noted that in the five specimens of *Loris* examined, 'The lacrymal appears neither inside the orbit nor on the face'. This is also true of *Nycticebus*. But in the closely related lemurs *Galago* and *Perodicticus*, a lacrimal is present. RAU and HIRIYANNAIYA (1931) also noted the absence of a lacrimal and agreed with GREGORY (1920) that the disappearance of the bone may probably be due to the shifting of the eyes and the shortness of the snout. LE GROS CLARK (1934, p.

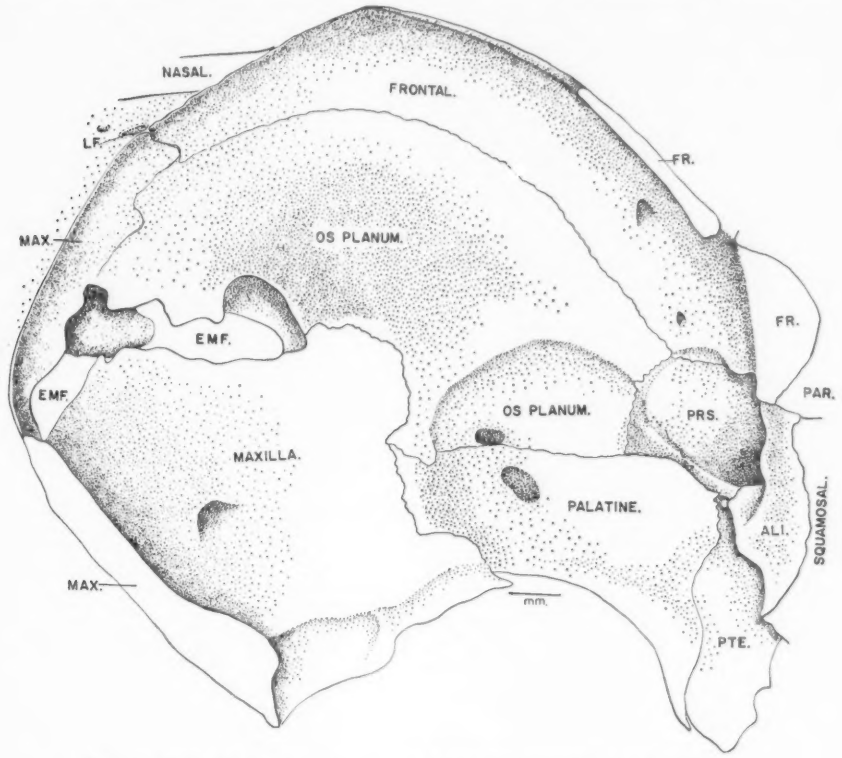

Fig. 31. The left adult orbit of *Loris* with the postorbital bar removed.
ali. alisphenoid; emf. ethmomaxillary fissure; fr. frontal; lf. lacrimal foramen; max. maxilla; par. parietal; prs. presphenoid; pte. pterygoid.

43) also recorded that the lacrymal was said to be absent in *Loris* and *Nycticebus*.

HILL (1933) examined both Ceylon and Mysore races of *Loris* and in describing the ethmomaxillary fissure, he noted that 'due to the union of the frontal, os planum, lachrymal and maxilla' at the anteromesial angle of the fissure, a cruciate arrangement of the sutures was noticed. Further, on the same page (p. 107) we read that 'The lachrymal bone in *Loris* is very small, due to the encroachment of the two orbits on the interorbital region. It is situated on the orbital margin and separated from its fellow by the two nasal bones'. Again he confirmed this by stating (1936, p. 292) that 'The lachrymal is very small, but never absent as stated by LE GROS CLARK (1934 b)'. This is the only author who describes the occurence of a lacrimal in *Loris**. As already said, the bone is *absent* in the family Lorisiidae (or Lorisidae) while some members of Galagidae are reported to possess it (FORSYTH-MAJOR, 1901). In the develop-

* See also Hill (1953, p. 139).

mental stages of *Loris* or in the adult skulls studied by me, a lacrimal was absent. That HILL was reporting an individual variation is also ruled out, for having examined a large collection of skulls, I have not noticed any variation in the topography of the bones of this region. I have drawn an enlarged view of the orbit (Fig. 31) of *Loris* to show the relative disposition of the bones. The os planum of the ethmoid forms the major portion of the medial wall anteriorly and between the os planum and the maxilla, there is a large gap (emf), referred to by HILL (1933) as the ethmo-maxillary fissure, which looks into the nasal sinus. Even in young skulls, the presence of this gap could be seen. In this connexion, the important correlation established by FORSYTH-MAJOR (1901) is very significant. He noted that 'In a general manner we can state that in those Lemuridae which are provided with a large lacrymal, the os planum is reduced; *vice versa* the lacrymal is reduced or absent—atleast from the orbit and the outer surface of the cranium—in those lemurs which are provided with a large os planum'.

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Usually the internal surface of the cranium of a Primate is studied under three heads: the anterior, middle and posterior cranial fossae.

The anterior cranial fossa comprises the cribriform plate and the anterior portion of the presphenoid. The cribriform plate is united with the ethmoid and along with the presphenoid posteriorly forms a trough. The plate shows a few large foramina for the passage of the branches of the olfactory nerves.

The presphenoid shows a pair of sinuses in this region.

As in the human skull, the middle cranial fossa (Fig. 32) is bounded anteriorly by the posterior edges of the parasphenoidal lesser wings (ors), posteriorly by the dorsum sellae (ds), posterior clinoid processes (pcp) and the superior border of the petrous part and laterally by the great wings of the basi-ali-sphenoids (gw-ali) and the squamous temporal (sqt).

In *Loris* the presphenoid bone is mostly separate from the combined basi-ali-sphenoids. I do not want to call the latter a 'sphenoid' as it would give an impression that the presphenoid is also united with it as has been done by RAU and SAHASRABUDHE (1930).

There is a groove leading into the optic foramen called sulcus choanalis, also noticed in the human skull. The optic foramen (Fig. 32, of) is noticed in the posterior wall of the presphenoid, bound ventrolaterally by the pila metoptica. The pila metoptica and the median pillar of presphenoid are not in the same line and therefore, there is a groove formation. Developmentally it is noticed that the orbitosphenoid ossifies both in front and back of the optic foramen in the pillars and unites with the presphenoid.

Behind the presphenoid suture with the composite basi-ali-sphenoids, posteriorly, there is a horseshoe-shaped depression for the hypophysis, the sella turcica (st). The elevation referred to as tuberculum sellae in the human skull is not discoverable and therefore, the entire depression in this region is the

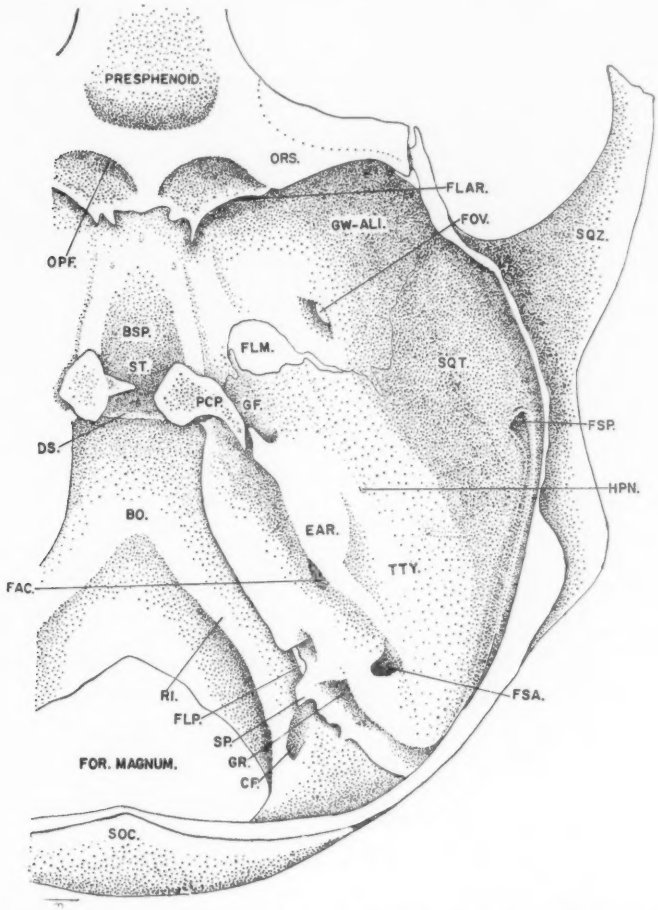

Fig. 32. Middle and posterior fossae of the adult skull of *Loris* with the roofing bones removed.

bo. basioccipital; bsp. basisphenoid; cf. condylar foramen; ds. dorsum sellae; ear. arcuate eminence; fac. acustic foramen (internal auditory meatus); flar. foramen lacerum anterius and rotundum; flm. foramen lacerum medium; flp. foramen lacerum posterius; fov. foramen ovalis; fsa. fossa subarcuata; fsp. foramen spinosum; gf. gasserian impression; gr. groove; gw-ali. greater wing of alisphenoid; hpn. hiatus for petrosal nerve; opf. optic foramen; ors. orbitosphenoid; pcp. posterior clinoid process; ri. ridge; soc. supraoccipital; sp. spine; sqt. squamous temporal; sqz. zygoma of the squamosal; st. sella turcica; tty. tegmen tympani.

fossa hypophyseos. Posteriorly to the fossa, there is the very delicate elevation of the dorsum sellae (ds) and the dorsolateral portions of it, near the cochlear capsule, project as hollow pillars whose upper extremeties spread irregularly; these are the posterior clinoid processes (pcp). There is a posterior process given off from each clinoid process which meets a process from the superior border of the petrous part.

In *Loris*, the lateral part of the fossa may be described as included between the greater wing of the basi-ali-sphenoids (gw-ali), the anterior region of the petrous part and laterally by the squamous temporal (sqt). This fossa and the orbit communicate with each other by the foramen lacerum anterius (sphenoidal fissure) which latter is bounded dorsomesially by the pila metoptica and dorsolaterally by the greater wing of basi-ali-sphenoids. This transmits the eyemuscle nerves, the ophthalmic nerve and vein and the maxillary branch of the trigeminal.

A separate foramen rotundum is absent; the maxillaris branch of the trigeminal nerve gains exit along with the eyemuscle nerves and probably the foramen rotundum is united with the sphenoidal fussure. RAU and SAHASRABUDHE (1930) also recorded that '. . the foramen lacerum anterius which, as mentioned above, is usually confluent with the foramen rotundum; whilst in some skulls the former is incompletely separated from the latter by a bony spicule'. I have not been able to observe this spicule in any skull studied by me.

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The foramen ovale (Fig. 32, fov) is lateral to the foramen lacerum medius; it is in the greater wing of the basi-ali-sphenoid bone. The third branch of the trigeminal nerve gains exit by this orifice.

In *Loris*, the foramen spinosum (Fig. 32, fsp) is noticed in the middle region of the temporal bone where the latter rises up as the squama and a bristle passed through this orifice gains exit by a large foramen posterior to the glenoid fossa (glf); the bristle passes through the tympanic cavity. RAU and SAHASRABUDHE (1930) have also described a post-glenoid orifice* transmitting meningeal vessels into the cranial cavity and have compared it with the foramen spinosum of the human skull.

The foramen lacerum medium (Fig. 32, flm) is situated between the posterior margin of the greater wing of basi-ali-sphenoids and the anteromesial border of the petrous temporal and is posteromesial to the foramen ovale (fov). This gaplike opening is noticed to extend laterally as a fissure and transmits the internal carotid artery. The occurrence of a foramen lacerum medium appears to be an unique feature of *Loris* skull. In *Lemur* and *Tarsius* and a number of monkeys, the carotid foramen passes through the periotic bulla or posteriorly to it; I do not agree with HILL's (1936) generalization that the Lorisoids show a foramen lacerum medium 'as in monkeys'.

In *Loris*, posterolaterally to the posterior clinoid process, there is a slight depression (Fig. 32, gf) representing the gasserian impression. RAU and SAHASRABUDHE (1930) also recorded that 'The apex of the petrous part of the temporal bone lateral to the posterior clinoid process shows a depression lodging the gasserian ganglion'.

The petrous temporal shows posterior to its contact with the posterior clinoid process an elevation, the arcuate eminence (ear) as in the human skull. It

* Hill (1953, p. 139) quotes that a postglenoid foramen is absent in *Loris*.

marks the region of the superior semicircular canal; laterally there is a small orifice (hpn) with a groove running towards the foramen lacerum medium. The orifice or hiatus is for the greater petrosal nerve.

Mesially to the squame of petrous temporal and laterally to the elevated superior border of it, lies the roof of the middle ear or tympanic cavity. This region is called tegmen tympani (tty).

The mesial wall of the petrous part shows certain elevations and orifices. Anteriorly there is a passage, the internal auditory meatus* or foramen acusticum (fac) in which there is a projection which separates a lower canal for the auditory nerve and a dorsal blind niche. Posterior to this is the large fossa subarcuata or parafloccular fossa (fsa). The elevation behind this marks the continuation of the superior semicircular canal from the arcuate eminence. In some skulls of *Loris*, ventral to the parafloccular fossa, there is also a large gap which is in continuity with the parafloccular fossa mesially, but separated by the bony eminence of the semicircular canal. In other skulls, this gap is closed up and a large groove (gr) is noticed at the ventral end of this. RAU and SAHASRABUDHE (1930, p. 184) in describing this region of the skull recorded that 'Immediately above jugular foramen is an elevation caused by the semicircular canal, and which leads into a fossa in the petrous bone'. They are obviously referring to the lower opening of the parafloccular fossa dorsoposteriorly to the foramen lacerum posterius; they have, however, not observed the occurrence in an equally large number of skulls, the other condition that I have described above, viz., the groove or an irregular depression below the semicircular canal elevation instead of a gap. Further, we read in their account on the same page that 'Posterior to this is a groove leading to an aperture which corresponds to the exit of ductus endolymphaticus of the human skull'. I have carefully examined a large number of skulls and I have failed to notice the aperture referred to above by the authors though the groove is seen in some. I do not think this could be an individual variation.

With regard to the foramen lacerum posterius (jugular foramen), RAU and SAHASRABUDHE (1930, p. 184) noted that 'The foramen lac. posterius is situated along the petro-occipital suture, and it is divided into two parts by a bony projection. The anterior part is slitlike and the posterior seen under cover of the bony projection appears round'. I am able to confirm this observation of the authors.

Posterior to the jugular foramen ventrally to the semicircular canal elevation bounding the fossa subarcuata, there is a depression in the mastoid portion, facing the similar one in the occipital bone. This is the sigmoid sulcus.

A description of the interior of the bulla and the disposition of the auditory ossicles is not given by RAU and SAHASRABUDHE (1930) and therefore, I am describing it in detail.

* Where the facial and acustic nerves run together.

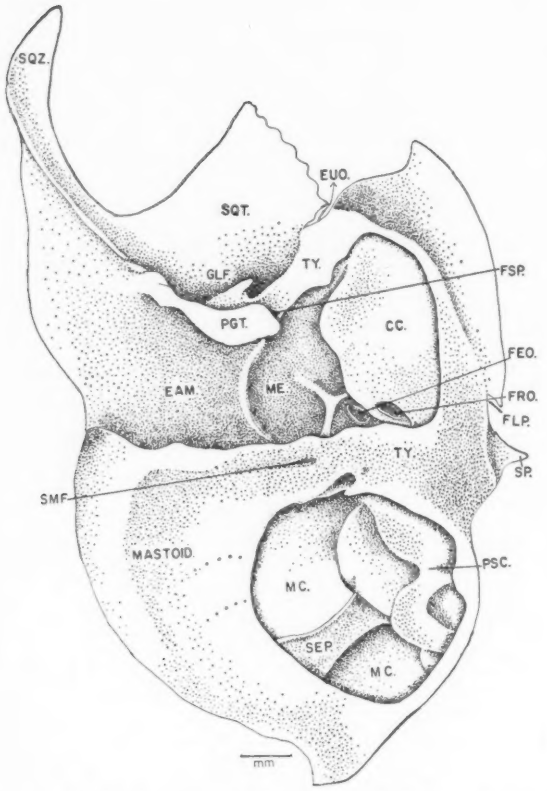

Fig. 33. Ventral view of the left temporal bone of adult *Loris*, with some parts removed to show the internals.

cc. cochlear capsule; eam. external auditory meatus; euo. position ofeustachian opening; feo. fenestra ovalis; flp. position of foramen lacerum posterius; fro. fenestra rotundum; fsp. foramen spinosum; glf. glenoid fossa; mc. mastoid cavity; me. middle ear; pgt. postglenoid tubercle; psc. posterior semicircular canal; sep. septum separating mastoid cavities; smf. stylomastoid foramen; sp. spine; sqt. squamous temporal; sqz. zygoma of squamosal; ty. tympanic part of periotic.

The tympanic bulla (auditory bulla: Wood Jones 1929) is a fairly large spherical chamber in *Loris*. Rau and Sahasrabudhe (1930, p. 179) described this region as 'more or less a rectangular projection'. According to Wood Jones (p. 97, Figs. 20, 21) in typical lemurine forms the tympanic bone is adherent to wall of the cavity. I have examined this point carefully and am able to confirm his observations. The tympanic is confluent with the wall of the bulla at the region where the external auditory meatus starts; in fact, the external auditory meatus is formed by the growth of the tympanic in the ventral region.

It should be pointed out in this connexion that Hill (1933. p. 105) in describing the temporal region of *Loris* skull stated that '.. all the Lorisoids agree in having the ectotympanic element lying outside the bulla. In this character

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they differ from all the Madagascar Lemuroids, where the bone is enclosed within the bulla. This fact was first ascertained by FORSYTH-MAJOR (1901) .. and POCOCK (1918)'. I have not come accross any reference to the above in Forsyth-Major's paper.

In my figure 33, a portion of the ventral wall of the external auditory meatus in between the stylomastoid and postglenoid tubercle and also a portion of the bulla wall is removed to expose the mastoid cavities. As pointed out by WOOLLARD (1925) and HILL (1933), the septum separating the middle ear cavity into an anterior and posterior portions is incomplete. The cochlear capsule is mesially situated and a median sulcus or groove demarcates the capsule into an anterior and posterior portion; the posterior portion shows a large oval opening, the fenestra rotundum (Fig. 33, fro). Dorsally to this posterior portion, there is the fenestra ovalis (feo) into which the (stapediform) stapes fits. Three blind niches can be observed between the ventral wall of the posterior portion and the wall of the bulla, in front of the septal wall.

There is a large passage connecting the anterior portion (tympanic) with the mastoid portion; this passage is dorsal to the capsule portion enclosing the three semicircular canals. Posteriorly in the spacious mastoid cavity, there is a ridge running along its wall. In this cavity the eminences for the external and posterior semicircular canals (psc) and the bony elevation accommodating the paraflocculus could also be made out.

The posterior cranial fossa is deeper than the other two and is bounded anteriorly by the dorsum sellae (Fig. 32, ds), laterally by the petrous-mastoid part of the temporal bone, and posteriorly by the elevated squame of the occipital bone (soc).

The floor of the fossa exhibits the large, almost rectangular foramen magnum. The occipital condyles are not visible from the dorsal aspect. From the dorsum sellae, the basilar part of the occipital (bo) forms a slope towards the foramen magnum and a prominent bony ridge (ri) on either side demarcates the petrous part of the occipital from the condylar portion. There is a clear petro-occipital fissure separating the basilar part of the occipital from the petrous temporale.

At about the region of the commencement of the ridge referred to above in the occipitopetrous fissure, is noticed the foramen lacerum posterius (flp) which however, is bounded posteriorly by a prominent spine (sp) given off from the petrous part sitting on the basilar part of the occipital. Posteriorly to the spine also, a small foramen is noticed. As already said, according to RAU and SAHASRABUDHE (1930) both these apertures form parts of the foramen lacerum posterius.

Below the bony ridge in the basilar part of the occipital, the hypoglossal foramen (anterior condylar, cf) is noticed. There is also another foramen posterior to the condylar (not seen in Fig. 32) which serves as a passage for the pharyngeal artery (RAU and SAHASRABUDHE, 1930).

In the squamous portion of the occipital, towards the foramen magnum are three depressions, the lateral of which face the sigmoid sulcus of the otic capsule.

DISCUSSION

After reviewing the structure and development of the chondrocrania of Primates, DE BEER (1937, p. 468) concluded that that of *Tarsius* resembles the Catarrhini more than the Lemuroidea. His study also emphasised 'the primitive nature of Primates'. FRETS (1914), working on the nasal capsule of Primates, has also come to the same conclusions. According to WEN (1930), however, the nasal capsule of *Macaca* appears to have been derived from the lemuroid type while that of *Galago, Nycticebus* and *Tarsius* is more Platyrrhine. I propose to compare the chondrocarnium of *Loris* with that of *Tarsius* and of the higher monkeys in order to ascertain whether this throws any light on the relationships of *Loris*. The interrelationships of Primates have been discussed by WOOD JONES (1929) and LE GROS CLARK (1934) from a study of the adult anatomical characters, and HILL (1936) has similarly discussed the affinities of lorisoids.

The general shape of the olfactory capsule of *Loris* differs from that of *Lemur, Nycticebus* and *Tarsius* (WEN, 1930). In *Nycticebus* and *Loris*, the lamina transversalis anterior is incomplete and a zona annularis is thus not formed; this is also the case in Catarrhini. In *Tarsius* and Platyrrhini, a zona is present. A lamina cribrosa through which the olfactory nerves pass is developed in *Loris, Nycticebus,* monkeys and man; in *Tarsius* there are two large orifices in the roof of the olfactory capsule for the olfactory nerves.

The posterior portion of the nasal region is slightly covered over by the orbital cartilages both in *Loris* and *Tarsius*; in *Nycticebus* and monkeys the orbital cartilage extends over the posterior wall of the nasal capsule considerably. In *Nycticebus,* Platyrrhini and Catarrhini (HENCKEL, 1928) and *Loris,* the sphenethmoid commissures enclose the foramina advehentia; in *Tarsius* the commissures are absent. The paraseptal cartilage is incomplete in *Loris* and *Nycticebus*. In *Tarsius,* it is probably complete (FRETS, 1914). In *Nycticebus* and *Loris* the basal plate is broad, while in *Tarsius* it is narrow and thus resembles other placental mammals. A steep fall in the basal plate from the dorsum sellae to the foramen magnum is seen in *Loris* and *Tarsius*; this incline appears to be absent in *Nycticebus* (HENCKEL, 1928).

A basicochlear fissure is absent in *Nycticebus* (HENCKEL, 1928) but feebly represented in *Loris;* it is quite broad in *Tarsius*. The ala temporalis is imperforate in *Loris, Nycticebus* and *Tarsius;* in the Platyrrhini and Catarrhini it is perforate. An alicochlear commissure is not developed in *Nycticebus, Loris, Tarsius,* Platyrrhini or Catarrhini.

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The above analysis makes it clear that *Nycticebus* and *Loris* show many similarities, which is in conformity with their classification in the same family. The absence of a basicochlear fissure in front of the jugular foramen in *Nycticebus* is probably a specialization. There are also important characters in which the chondrocranium of *Loris* differs from that of *Tarsius*: 1) the possession of a broad basal plate; 2) the possession of a sphenethmoid commissure, 3) the absence of an orbitoparietal commissure, and 4) the absence of a complete zona annularis. If *Loris* and *Tarsius* were closely related, such differences could hardly be expected. In all except the first of the characters just mentioned the chondrocranium of *Loris* resembles the higher Primates.

As already noted, FRETS (1914) and DE BEER (1937) concluded that *Tarsius* resembles the Catarrhini more than the Lemuroidea. Judging from their description, however, the chondrocranium of *Tarsius* differs from that of the Catarrhini in the 1) absence of a sphenethmoid commissure, 2) presence of an orbitoparietal commissure, and 3) presence of a zona annularis. *Tarsius* also differs from the Platyrrhini in the first two characters. It is doubtful, therefore, as HENCKEL (1928) observed, whether the chondrocranium of the higher Primates could be derived from the tarsioid type.

DE BEER (1937) compared the chondrocranium and the bony skull of the Primates with those of the rabbit and came to the important conclusion that the latter shows a number of primitive features. In comparing *Loris* chondrocranium with that of rabbit, it is noticed that *Loris* differs from the latter in at least four of these primitive features: 1) in possessing incomplete paraseptal cartilages, 2) in the absence of mastoid and paracondylar processes, 3) in the absence of the ala hypochiasmatica, and 4) in the absence of the orbitoparietal commissure. Obviously *Loris* stands apart from the rabbit in all these features.

Let us now examine if the adult skull of *Loris* throws any light on its interrelationship. WOOD JONES (1929) and particularly LE GROS CLARK (1934) have referred to this aspect of the problem in their studies of Primates and recently, HILL (1936) has referred to this in discussing the affinities of Lorisoids. Therefore, I shall refer here briefly to the same in view of the developmental study I have made.

1. In lemurs and lorises, the frontals form more the front wall than the roof of the cranium posteriorly; in *Tarsius,* Platyrrhini and Catarrhini, the frontals contribute more of the roof posteriorly.

2. The frontal descends to meet the palatine bone in lemurs as there is no os planum of the ethmoid; in *Loris* the os planum separates the frontal and palatine bones. In *Tarsius* the os planum is anteriorly disposed and the frontal and palatine bones come together posteriorly to the ethmoid. In Platyrrhini and Catarrhini the os planum and sphenoid meet so as to separate the frontal and the palatine bones.

3. In the nasal region of *Loris* there are three ethmoturbinals (four accord-

ing to HILL, 1936) and a scroll-like maxillo-turbinal; in *Tarsius* the maxillo-turbinal is attenuated and it has only two endoturbinals.

4. In the lemurs the jugal extends into the orbital border and meets the lacrimal, keeping out the maxilla from the orbit; the lacrimal, however, does not meet the nasal. In *Loris,* as there is no lacrimal, even though HILL (1936, 1953) affirms to the contrary, the maxilla forms the anterior border. In *Tarsius,* the jugal does not extend to meet the lacrimal and therefore, a small portion of the maxilla is included in the orbital border; the lacrimal just touches the nasal. In the Platyrrhini the lacrimal is large and since the maxillary process of the jugal is short, the maxilla forms the anterior border of the orbit; the lacrimal meets the nasal. The frontal and maxilla in front of the lacrimal do not meet in lemurs, *Tarsius* and Platyrrhini; in *Loris,* as there is no lacrimal, the frontal contacts the maxilla. In Catarrhini even though there is the lacrimal behind the nasal, the frontal and maxilla meet as also behind the lacrimal separating the lacrimal from os planum.

5. The posterior extension of the jugal to the front of the glenoid fossa is considered a primitive feature. In *Tarsius,* the jugal does not extend to the temporomandibular articulation; so also in *Hapale* (WOOD JONES, 1929) and the Catarrhini. In *Loris* also the same condition is met with.

6. In lemurs the nasals are broad posteriorly; in *Loris* and *Tarsius,* the bones become narrower posteriorly and in the Catarrhini, there is a median nasal (formed by the union of the two bones) with the frontal end exceedingly narrowed.

7. The orbital process of frontal which meets the jugal is large in lemurs and *Loris;* in *Tarsius* it is very short. In the two former genera, on account of the large orbito-frontal bar, the jugal is widely separated from the cranial wall. In *Tarsius,* on the other hand, a portion of the postorbital bar comes in contact with the cranial wall to form a postorbital wall. In *Hapale* the jugal-parietal contact forming a postorbital wall is so great that it 'excludes the alisphenoid from contact with the frontal upon the lateral cranial wall' (WOOD JONES, 1929). In the Catarrhini, the jugal never comes in contact with the parietal, the sphenoid and squamous temporal separating it.

8. In the lemurs, the parietal contacts over a wide area the sphenoid; so also in *Tarsius.* In *Loris,* it is a narrow contact. In Platyrrhini the contact is quite wide but in the Catarrhini this contact is lost since the squamous temporal intervenes.

9. The tympanic bone lies free in the auditory bulla in lemurs, while in *Loris* and *Tarsius* it is fused with the outer wall. So also in Platyrrhini and Catarrhini. In *Loris, Tarsius* and the Catarrhini, the external auditory meatus is formed by the tympanic; the contribution is very little in the case of Platyrrhini.

The above analysis leads one to conclude that there are atleast five features (items 2, 4, 6, 8, 9, of the above list) in which *Loris* differs from lemurs and

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quite a number in which *Loris* differs from *Tarsius*. On account of the differences between *Loris* and lemurs, I agree with HILL (1936) in stating that the families Galagidae and Lorisidae should no more be treated under Lemuroidea and that the suborders Lorisoidea and Lemuroidea should enjoy the status equal to that of Tarsioidea.

There are atleast seven features in which *Loris* stands apart from *Tarsius*, viz., 1) the little participation of the frontals to form the roof, 2) the os planum separating the frontal and palate, 3) the absence of a lacrimal, 4) the large orbital extension of frontal, 5) the narrow contact between alisphenoid and parietal, 6) the development of a foramen lacerum medium for the passage of the carotid artery, and 7) the large maxilloturbinal and three ethmoturbinals. *Tarsius*, coming as it does under the haplorhine series and showing so many differences cannot be imagined to be related to either *Lemur* or *Loris* and Le Gros Clark (1934) rightly pointed out that the 'skull of the ancestral tarsioids could hardly be referred either to the lemuriform or lorisiform group of the Lemuroidea...'. The view of HILL (1936) that the Lorisoids have more in common with the Tarsioids than with Lemuroids is not substantiated by my study of *Loris* skull.

On comparing *Tarsius* with Platyrrhini, one notices points of similarity. The formation of the postorbital wall by the jugal coming in contact with the lateral wall is also seen in Platyrrhini, only in the latter the parietal-jugal contact is greater. The alisphenoid-parietal contact is also broad in both. The lacrimal comes in contact with the nasal in both *Tarsius* and Platyrrhini and thus the fronto-maxillary joint is abolished. In all these features, *Tarsius* differs from the Catarrhini. WEN (1930) also pointed out that in the nasal cartilages *Tarsius* resembled more the Platarrhini. There appears to be therefore, greater similarity between the nasal capsule and bony skull of the Platyrrhini and of *Tarsius*.

Comparing *Loris* with higher Primates, it is noticed that it resembles them in: 1) the presence of an os planum, 2) the maxilla forming a portion of the front wall of the orbit with the lacrimal foramen outside it, 3) the shortness of the squamous extension of the jugal, 4) the ectotympanic forming the outer wall of the bulla, 5) having only three ethmoturbinals and 6) the narrowness of the nasals towards the frontals. *Loris*, however, also shows certain differences from the higher Primates in that 1) the frontal forms more of the front wall of the roof, 2) a lacrimal is wanting and 3) the dentition is triconodont.

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I am deeply indebted to Professor DIETRICH STARCK, Director, Schenkenberger's Anatomical Institute, Frankfurt on Main, for his kindness in making

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SUMMARY

1. The development of the chondrocranium and the appearance of the centres of ossification during development in *Loris* and its adult skull have been studied with a view to examine its relationship with the other Primates.

2. The fully-formed chondrocranium discloses the following features: the basal plate shows a steep incline towards the foramen magnum and the side view of the chondrocranium discloses the relative levels of these; the occipital arches are united posteriorly with the basal plate, showing a prominent ridge. Dorsally, a tectum posterius connects the occipital arches. The otic capsules are surmounted by the parietal cartilages and these are *not* connected with the planum supraseptale by the orbitoparietal commissures. The otic capsule is connected mesially with the basal plate by the basivestibular and sphenocochlear commissures.

There is a prominent dorsum sellae; in front of this in early stages (6.5 mm), a hypophysial fenestra is noticed. In the fully-formed chondrocranium the hypophysial fenestra is obliterated and laterally, the processus alaris is noticed. A processus pterygoideus and a broad ala temporalis are noticed; the latter is imperforate.

The pilae metopticae and prooticae are broad and the orbital cartilage is connected with the roof of the nasal capsule, laterally to the foramen olfactoria advehentia, by the sphenethmoid commissure.

Three sulci, the anterior, posterior and the dorsal demarcate the olfactory capsule. The epiphanial foramen is fissure-like. The lamina transversalis anterior is incomplete and there is no complete zona annularis. The nasolacrimal duct opens between the lamina transversalis anterior and a projecting cartilage from the ventromedian wall of the cupula anterior. The lamina transversalis posterior is broad and encloses Jacobson's organ. From the floor of the lamina orbitonasalis, there projects forwards the posterior paraseptal cartilage unconnected with the median septum nasi or with the anterior paraseptal cartilage. At the region where the sulcus anterior separates the anterior and middle portions of the nasal capsule, there is an independent piece of cartilage laterally to the cranial wall.

There are five sets of turbinals: the atrioturbinal, the nasoturbinal, the frontoturbinal, the maxilloturbinal and three ethmoturbinals.

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In the visceral skeleton, Meckel's cartilage shows posteriorly the malleus with its manubrial process, incus with its two processes and the stirrup-shaped stapes.

3. The dentaries and maxillae are the first to appear (8 mm) in the osteocranium. In the next stage (11 mm) the premaxillae, frontals, parietals, jugals, palatines, squamosals and only one cartilage bone, the basioccipital have appeared. In the 12 mm stage, the nasals, paired prevomers, pterygoids, exoccipitals and the supraoccipital have appeared. In the next stage (15 mm), the prevomers have fused, the basisphenoid is noticed to arise as three centres of ossifications and the pterygoid has fused with the membranous portion of the alisphenoid, which bone has also appeared; prearticulars and orbitosphenoids are also seen at this stage. In the 20 mm stage, the presphenoid, prootics and opisthotics are seen, the latter two in continuity. In the next stage (25 mm), the prearticular has fused with the malleus, the incus and stapes are ossified and the orbitosphenoid has fused with the presphenoid. In the 27 mm stage, the alisphenoid wing is extending to meet the squamosal. In the 35 mm stage, the turbinals have appeared in the olfactory sac, the basisphenoid has fused with the alisphenoid and the periotic with the tympanic and squamosal.

4. The study discloses that the chondrocranium of *Loris* resembles that of *Nycticebus* and differs from that of *Tarsius*. *Tarsius* chondrocranium also shows points of difference from that of the Catarrhini. In the bony skull, *Loris* shows atleast five features in which it differs from *Tarsius* but resembles the higher monkeys, and *Tarsius* is noticed to resemble the skull of the Platyrrhini.

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THE FIBRILAR SYSTEMS OF LAMELLIBRANCH EPITHELIAL CELLS AS REVEALED BY ARGENTIC IMPREGNATION

BY

*EMILIO PALAFOX**

INTRODUCTION

The epithelial ciliated cells of the molluscs, especially those of the lamellibranchs, have always been the preferred material for the study of the ciliated cell's general morphology. The existence of the ciliary rootlets has been known since the works of FRIEDRICH (1858), while the works of ENGELMANN (1880) and later authors indicated its conical arrangement as well as the existence of the other components of the ciliary system.

HEIDENHAIN (1899) described in the epithelium of the gastropods a reinforcing system of fibrils, which he called *tonofibrils*. Later RIO-HORTEGA (1917a) described in different epithelia, fibrilar formations which he called *epitheliofibrils*, utilizing original methods of silver impregnation. Under this designation he included true tonofibrils (in the digestive epithelium of *Helix*, as an example), as well as ciliary rootlets (in the hepatic ducts of *Helix*, previously noted by ELLERMANN in 1899, and in the digestive apparatus of *Tapes*). Since then the tonofibrils of the epithelial cells have been known as epitheliofibrils (ORTIZ PICÓN, 1947).

The details of the structure of the molluscan digestive epithelium have been studied among others by VIGNON (1901) in *Pecten, Mya* and *Doris;* by KOLACEV (1910), ERHARD (1910) and GUTHEIL (1911, 1912) in *Anodonta;* by MACKINTOSH (1925) in the gastropod *Crepidula*; and by GRAHAM (1931, 1932) in *Ensis* and *Patella*, respectively. Recently, WORLEY (1940, 1941) investigated the structure of the ciliary cones of the lamellibranchs and the ciliary activity of the intestinal epithelium of *Anodonta, Sphaerium* and *Venus*, and PALAFOX (1952) reported on the structure of the same epithelium in *Tapes*.

In this work and in an effort to review the structure of the ciliated epithelial cells as revealed by argentic impregnation, I have studied the fibrilar systems of the digestive and tegumentary epithelia of *Tapes (Paphia) decussatus* (L.). It was begun in the laboratories of the Centro de Investigaciones Zoológicas

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(of the C.S.I.C.), in Madrid, Spain, and was concluded in the Centro Dermatológico, Culiacán, Sin., México. I wish to express my appreciation to the Directors and personnel of these Centers, who generously offered me the use of their facilities. It is also a pleasure to manifest my gratitude to Dr. FRITZ HAAS, of the Chicago Natural History Museum, Chicago Ill., for his kind aid.

MATERIALS AND METHODS

The intestinal and cutaneous epithelia of *Tapes (Paphia) decussatus* (L.) was studied in fresh specimens obtained from the North Athlantic coast of Spain, fixed in Bouin's fluid or in neutral 10 % formalin. The fixation was made *in toto* or only in the digestive apparatus previously dissected.

Previous to inclusion in paraffin according to Peterfi the material fixed in Bouin's fluid was stained with Heidenhain's iron hematoxylin in serial cuts of 6 to 8 microns. From the material fixed in formalin were obtained frozen sections 10 microns in thickness which permitted the application of the gold-silver impregnation techniques, especially the first three variants of RIO-HORTEGA (1916a) to the Achúcarro's tannin-silver method; RIO-HORTEGA's (1917b, 1926, 1942) silver carbonate methods, with and without pyridin; FERNÁNDEZ GALIANO'S (1934) methods A and B based on the previously cited variants of RIO-HORTEGA.

In the use of the silver staining techniques, special attention should be given to the general observations given by W. PANFIELD and W. V. CONE in Mc Clung's Handbook[1]. Here one may also find indications regarding Rio-Hortega's silver carbonate methods, Achúcarro's tannin-silver method, and Rio-Hortega's variant one and four. The third variant—not indicated by Mc Clung—differs from the first in that the first variant uses a 1 % alcoholic tannic acid solution instead of a 3 % water solution.

Fernández Galiano's methods differ from the Rio-Hortega's variants in the following points: (a) In *method A* a 0.5 % tannic water solution is used as mordant, at room temperature for 10 minutes. The impregnation is made in a single dish containing 10 cc. of distilled water and 2 or 3 drops of Bielschowsky's amoniacal silver until the sections have acquired a pale yellow color. The sections are then immediately reduced with 20 % formalin. (b) In *method B* the same procedure is followed as in method A, but the sections are allowed to reach a dark yellow color in the silver solution. After washing and without reduction, the sections are passed through the 0.2 % gold chloride solution until they acquire a violet color. Then they are directly passed to 5 % hyposulphite of soda, washed, dehydrated, cleared, etc. as in the other metallic methods.

Although all of these methods were found difficult to apply to the different

[1] Mc Clung's Handbook of Microscopical Technique, New York, 1950, pp. 413 and ff.

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materials, and the silver carbonate with pyridin gave variable results, they all showed special selectiveness. Their constant and successive use in the same material, resulted in the manifestation of delicate and beautifully stained structures which the use of iron hematoxylin had not revealed or had barely suggested.

OBSERVATIONS

A. IN THE INTESTINAL EPITHELIUM

The cells of the intestinal epithelium of *Tapes*—one-layered as is the general case in the invertebrates—belong in their majority to the ciliated type, the remaining to the mucus secreting cells. The larger number of the ciliated cells are vibratory, and of these, six clearly distinct morphological types exist:

(1) Those that cover the major part of the intestine, show the common ciliated apparatus described by authors for the lamellibranchs. They are high prismatic cells (Fig. 1) having two rows of granules in the distal end. From the upper row, made up of the *bulbs*, arise the cilia, one for each bulb; from the lower, made up of the *basal granules*, arise one *ciliary rootlet* per granule. The *intermediate segment* unites each bulb with its corresponding basal granule found in an inferior position. The ciliary rootlets (*Wimperwurzeln* of ENGELMANN) converge towards their lower end, (Fig. 1 and 3) and form a cone (*Engelmann's cone*), from whose apex arises a fibril which passes near the nucleus. In this type of epithelium the ciliary cones obtain an aproximate length of 10 microns (in stomachal cells 90 microns in total length), but the technique utilized for its study—prolongated fixation in formalin and staining with pyridinated silver carbonate—did not permit the observation of the parallel condition of the first segment of the rootlets, in the manner indicated by WORLEY (1941).

(2) There is in the stomach a specialized epithelium whose function consists in the secretion of the gastric shield. Its cells bear stereocilia (Fig. 4), an observation which contrast with that of GUTHEIL (1912) in his studies with *Anodonta*, corroborated by NELSON (1918). Since these authors believe that this epithelium is devoid of cilia, then, following previous authors, they consider this region as the only one devoid of cilia in all the alimentary tract. YONGE (1926) writes in regard to *Ostrea* of " fine strands having the appearance of cilia and arising from basal granules". In the same region of the alimentary tract of *Tapes* I have not found basal granules, but I did find stereocilia and terminal bars (Fig. 4). The images of the ciliary apparatus, obtained with the silver carbonate, leads one to think that there exists in *Tapes* a small cone from which the stereocilia arise without the apparent presence of basal granules, and that these stereocilia are fixed in the gastric shield.

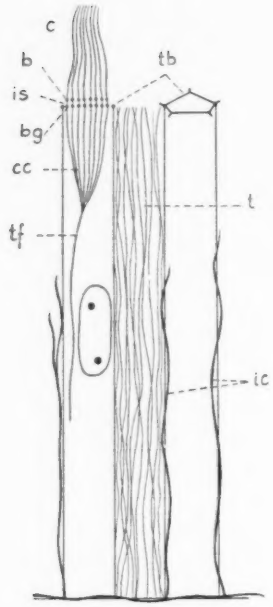

Fig. 1. Structure of the ciliary epithelial cell of *Tapes (Paphia) decussatus* (L.): b, bulbs; bg, basal granules; c, cilia; cc, ciliary cone; ic, interepithelial connective; is, intermediate segment; t, tonofibrils; tb, terminal bar; tf, terminal fibril. (Schematic).

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(3) In the style-sac region—*Magendarm* of the german authors—the epithelium, whose function is to set the crystalline style in a rotary and progressive movement, consist of prismatic cells whose length varies from 45 to 80 microns, the shorter ones being most abundant. Their ciliary system is characteristic (Fig. 6). Up to now it had been described by various authors as made up of ciliary rootlets, parallel and interrupted at a constant height. It is thus described by GUTHEIL (1912) in *Anodonta,* by RIO-HORTEGA (1917a) in *Tapes* and by NELSON (1918) in *Lampsilis.* It shows up in this manner in my preparations of *Tapes* stained by hematoxylin and in general by the silver techniques; but with the pyridinated silver carbonate the rootlets are stained along the known parallel section, which is about 16 microns in length, and it also shows up a short section of about 4 microns which is slightly convergent. It is more highly stained and is continued by lighter parallel ciliary rootlets which extend to the base of the cell. These ciliary rootlets do not constitute a cone. They correspond to strong cilia of about 16 microns in length and their special morphology is related to a characteristic ciliar function.

(4) The ciliary apparatus of the cells of the intestinal tract which segregate the crystaline style (Fig. 5) remind one of the stomach cells which intervene in the secretion of gastric shield. It was possible to stain, by means of Rio-Hortega's third variant, the cilia as well as the ciliary cones. Each cilia is prolonged into a rootlet without showing the presence of a basal granule, while in the adjacent regions of the same preparations these granules show up per-

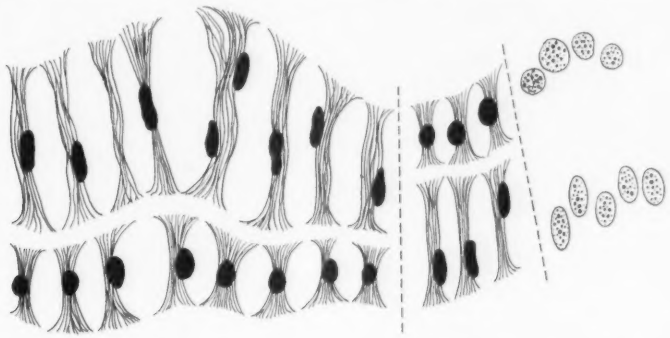

Fig. 2. Fibrils of the two-layered tegumentary epithelium (cutaneous and of the mantel) of *Tapes (Paphia) decussatus* (L.). The upper layer corresponds to the mantel and the lower to the tegument. At the right side appear the nuclei of the two-layers before they constitute a double layer. (Of the same preparation, stained with the III technique of Rio-Hortega's silver carbonate. About × 1500).

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fectly stained. It is possible to distinguish a distinct shading in the staining of the cilia, of its rootlet as well as of the transition area. This staining technique failed to differentiate the terminal fibril of the cone in this zone. It should be noted that ESCHRICH (1931) in his study of the homologous region in *Cultellus* asserts that its cells lack cilia, an assertion which greatly helps his interpretation of them as secreting cells.

(5) In the distal end of the rectum, in the region adjacent to the anal aperture, the epithelium forms slender waves of great length and the greater part of its ciliated cells shows a distinct morphology from those already described. They are small cells, 10 to 15 microns in length. Their cilia form individual tufts of great length since they reach a length several times that of their cells. They are observed in an undulating position in the sections (Fig. 7).

In these cells, Rio-Hortega's first variant shows up the presence of the double row of granules—bulbs and basal granules—while Fernández Galiano's method A stains the cilia and the bulbs, in addition to the chondriosomes. Method B of the same author stains the chondriosomes, the cilia and the basal granules, but not the bulbs. With the iron hematoxylin the ciliary rootlets are barely discernible in these cells while the terminal bar is well stained.

(6) It is possible to observe in sections of the digestive diverticula small cells with very long cilia whose existence is presumed by YONGE (1939) in previous observations in the living digestive diverticula of lamellibranchs. Rio-Hortega's third variant shows in these cells the double row of granules basal to the cilia.

Fig. 3.

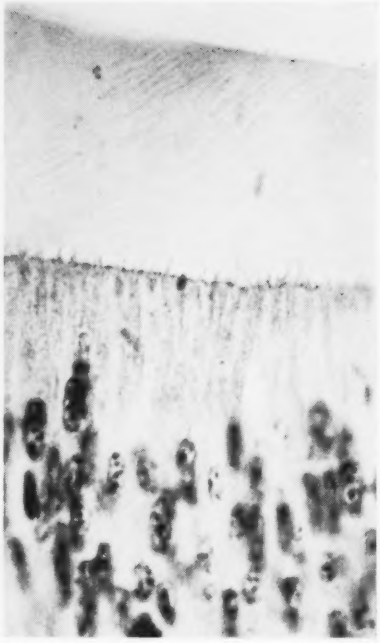

Fig. 4.

Fig. 3. Ciliary cones of the type 1 epithelial cells of the stomach. (Photomicrograph of a preparation stained with silver carbonate with pyridin. Oil immersion, × 1140).

Fig. 4. Type 2 epithelium of stomach—gastric shield zone—showing stereocilia. Lightly discernible, the terminal bars. (Iron hematoxylin. Oil immersion, × 1140).

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Fig. 5.

Fig. 6.

Fig. 5. Type 4 epithelium which secretes the crystaline style. The ciliary rootlets are barely discernible. (Stained by Rio-Hortega's third variant. × 540).

Fig. 6. Ciliary rootlets of the type 3 vibratil cells of the style-sac region. (Stained with silver carbonate with pyridin. Oil immersion, × 1140).

Fig. 7.

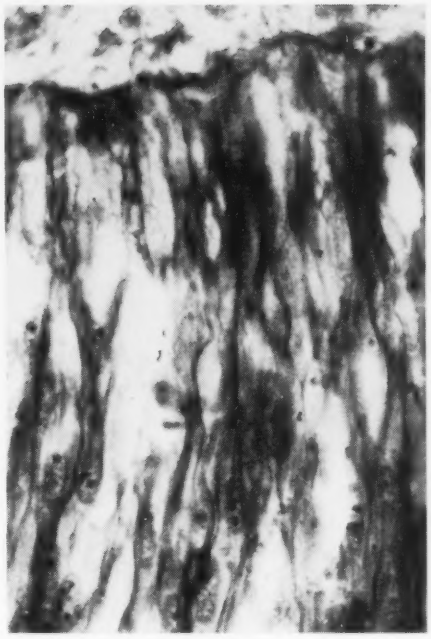

Fig. 8.

Fig. 7. Type 5 epithelial cells of the rectum. (Stained by Fernández Galiano's method A. × 1100).

Fig. 8 and 9. Tonofibrilar system of the upper epithelium of the stomach. The two photomicrographs show the distal and proximal ends of the cells of such epithelium, its length is greater than the field of both photomicrographs. The nuclei and nucleoli are also stained. (Silver carbonate with pyridin. Oil immersion, × 1140).

Fig. 10. Tonofibrils of the mid-gut epithelium. (Silver carbonate with pyridin. Oil immersion, × 1140).

Fig. 9.

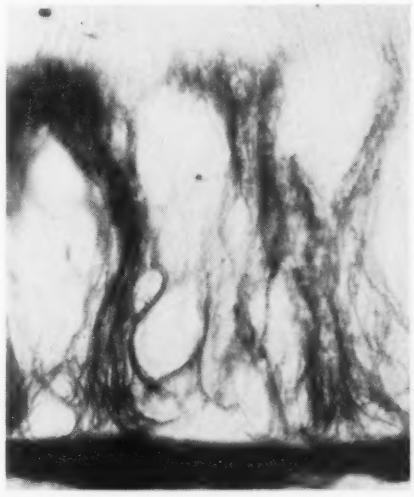

Fig. 10.

Tonofibrils

Staining with pyridinated silver carbonate shows a system of tonofibrils totally independent of the ciliary rootlets and the basal granules of cilia. They have been studied in detail in the cells of the stomach (Figs. 8 and 9) and of the mid-gut (Fig. 10). They are fibres which follow a wavy course and run from one end of the cell to the other having a reinforcing function. Those of the mid-gut show a stronger staining along the base of the cell and a lighter one in the distal extreme. When the system of tonofibrils is stained, the ciliary system remains without color. However, there are times when the ciliary system is stained and the tonofibrils show up lightly along the base of the cells, but it is always evident that these structures are independent of each other morphologically and in their function.

Interepithelial connective

Rio-Hortega (1916b) described, through the use of his *second variant*, a reinforcing structure of the epithelium which he designated as interepithelial connective. By using the technique known as Fernández Galiano's method B, I have observed in the terminal gut of *Tapes* fibres which are similar to those described by Rio-Hortega in other invertebrates (Fig. 1). They are conjunctive fibres which arise from the basal membrane and run upwards among adjacent cells of the epithelium, reaching close to the distal end. They are wavy and at times branched fibres.

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B. IN THE CUTANEOUS EPITHELIUM

Using the *third variant*, in *Tapes* tegument is observed a ciliated epithelium with the same characteristics as those described for *type 1* of the intestine. It also shows the common cutaneous epithelium of small cells, extremely short, non-ciliated, but provided with a cuticular brim. Transition from one epithelial type to another can be observed in the labial palps, whose two walls, outer and inner, are formed respectively by the ciliated and cutaneous epithelium.

The pyridinated silver carbonate method in its original manipulation (Rio-Hortega, 1926, 1942) allows one to study in this epithelium a typical tonofibrilar system, constituted by abundant fibrils which run across the cell in a longitudinal manner. Fibrils keep neither visible relations with the basal granules, nor with the epithelial basal membrane.

In the zone in which cutaneous epithelium joins with the mantel epithelium, they form a two-layered epithelium whose morphology is known.

Using his *third variant* Rio-Hortega (1917a) describes specially the particular disposition of its epitheliofibrils.

In contrast to these tonofibrils of intestinal and tegumentary epithelia, which were only observed by impregnation with pyridinated silver carbonate, fibrils

of the double epithelial layer (cutaneous and of the mantel) were stained by several non selective argentic methods which brought out other structures. For example, with the *III method* of silver carbonate (Rio-Hortega, 1942) the two-layered epithelium fibrils are clearly stained (Fig. 2) while the cutaneous and the mantel's epithelia show only the nuclear cromatine granula stained. The double epithelium nuclei appear then intensely stained as a compact mass. Its cells appear neatly, insulated one from the other. Epitheliofibrils are narrow in their middle and wider in their extremes, having a configuration similar to an hour-glass (Fig. 2). It is certain that these fibrils are of a different type from those of the intestinal and cutaneous epithelia.

DISCUSSION

By the systematic use of several gold-silver impregnation techniques it is possible to distinguish the following structures:

Structures	*Stainings*
Ciliary rootlets of the common ciliated epithelium ..	Ag_2CO_3 + Pyridin,* Rio-Hortega's third variant, Iron hematoxylin, Without staining.
Terminal fibril of cones	Ag_2CO_3 + Pyridin.
Ciliary rootles of the style-sac epithelium:	
(a) Parallel segment	All argentic methods, Iron hematoxylin.
(b) The whole rootlets system	AG_2CO_3 + Pyridin.
In type 1 and 5 cells:	
Cilia, bulbs and basal granula	Rio-Hortega's first variant.
Only in type 5 cells:	
(a) Chondriosomes, cilia and bulbs	Fernández Galiano's method A
(b) Chondriosomes, cilia and basal granules	Fernández Galiano's method B
Intestinal and cutaneous epithelia tonofibrils	Ag_2CO_3 + Pyridin.
Epitheliofibrils of the two-layered epithelium (cutaneous and of the mantel)	Rio-Hortega's III technique, Rio-Hortega's third variant, and other argentic methods.
Interepithelial connective	Fernández Galiano's method B

* Abbreviation amoniacal silver carbonate method with pyridin, using it such as it was done in its original form (Rio-Hortega, 1926).

Notwithstanding the fact that since RIO-HORTEGA proposed the term *epitheliofibril* it has been used mainly as a synonym for *tonofibril of epithelial cell,* it is evident that this is not accurate. Epitheliofibrils are not only the true tonofibrils of the epithelia, in which they have a reinforcing mechanical function, but are also ciliary rootlets, fibrilar differentiations of the distal cytoplasm, responsible for the coordination of the ciliary beat. Under the term epitheliofibrils also fall the two-layered epithelium fibrils, which although they may seem tonofibrils of different rank than those of the ordinary epithelia, their relations to the basal granules situate them nearer to the ciliary rootlets. *Epitheliofibrils is therefore a generic term valid for all intracytoplasmic fibrilar formations of the epithelium, without any regard to their possible function.*

The system of ciliary rootlets described for the vibratile cells of the style-sac, already previously stated as differing from the descriptions reported by other authors, is otherwise very much like the one described by MACKINTOSH (1925) in *Crepidula.* This author observes that ciliary rootlets run through the cells from one end to the other in a similar manner to the one already described in *Tapes.* However MACKINTOSH also points out that in *Crepidula* only few granules have ciliary rootlets and many only have cilia. This characteristic does not seem to occur in *Tapes,* in which all granules have ciliary rootlets, a fact which seems to be more in accordance with the function of the ciliary rootlets in their coordination of the ciliary movement (WORLEY, 1941).

In *Tapes* epithelia, nothing is found like the "intra-epithelial canals" described by MACKINTOSH in *Crepidula* and confirmed by YONGE (1926) in *Ostrea* and by GRAHAM (1932) in *Patella,* and whose absence GRAHAM (1931) points out in *Ensis.* MACKINTOSH assigns to these canals the nature of connective tissue fibres and YONGE a reinforcing function which makes possible the functioning of the cells of the style-sac, but both have doubts as to whether these canals pass through or between the cells. GRAHAM asserts that the nature of the structure studied in *Patella* remains doubtful, based on the negative results obtained by the use of specific colorants and by Bielshowsky's silver, and he also observed that the above mentioned canals, "... May not, therefore, be strictly named "intra"-epithelial canals, as they lie rather in the subjacent connective tissue than in the epithelium itself".

It is possible that the structures described by these authors are not homologous. If the structures described by Mackintosh and Yonge really have a conjunctive nature with an intercellular disposition and reinforcing function, then they may be included in the so-called *interepithelial connective* of Rio-Hortega, described in *Tapes,* in this work, by argentic impregnation as a fibrilar system of its epithelia.

If the disposition of the canals were intracellular, they could be related to those described by Cajal in the intestinal cells of *Hirudo* and by Sánchez in

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the intestine of several isopods. These canals have there origin in the conjunctive tissue and terminate in the cytoplasm (See: SÁNCHEZ, 1904).

Finally, importance should be given to the fact that the cells in the region of the gastric shield (cells type 2) do not have bulbs and basal granules but have stereocilia, and that the cells of the gastric intestine (type 4), probably with a secreting function, also lack bulbs and basal granules and are provided with non-motile cilia. It is known from the papers of PETER (1899), GRAY (1928) and others that the basal granules are responsible for the ciliary beat. The absence of granules in the above mentioned regions would be justified by the lack of function.

SUMMARY

The structure of the intestinal and tegumentary epithelia in *Tapes (Paphia) decussatus* (L.) is studied by means of the gold-silver impregnation techniques. Three different fibrilar systems are described: (1) ciliary rootlets; (2) tonofibrilar system; (3) fibrils of the so-called interepithelial connective. The first two are differentiations of the cytoplasm, and therefore intracellular. The last system is intercellular and of conjunctive nature. Several ciliary rootlets are distinguished: common ciliary rootlets; ciliary rootlets of the "style-sac" cells; ciliary rootlets of the streocilia in the gastric shield zone; those of the cells secreting the crystaline style. A differentiation is also made in the study of the tonofibrils. The tonofibrils of the two-layered tegumentary epithelium, of the mantel and of the body, constituted a special type.

The behavior of the elements of the ciliary system, of the tonofibrilar system and of the interepithelial connective varies with different staining techniques used. This eliminates all possible confusion in distinguishing these various elements.

The ciliary system has a motil function in most of the intestinal cells, and the tonofibrilar and connective systems are reinforcing structures for all the cellules. The three systems are independent of each other.

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THE ONTOGENY OF THE CRANIAL BONES, CRANIAL PERIPHERAL AND CRANIAL PARASYMPATHETIC NERVES, TOGETHER WITH A STUDY OF THE VISCERAL MUSCLES OF *STRUTHIO*

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(With 80 text figures and 11 plates)

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ABSTRACT

The skull of *Struthio* is typically avian. There are, however, many cranial features that are neotenic in relation to the other *Dromaeognathae* and *Neognathae*. The premaxillary-vomer arthrosis is present in the embryo of *Struthio* but is absent in the adult; it is present in the adults of the other *Dromaeognathae;* the trabeculo-capsular entity is uninterrupted: therefore, there is no mesokinetic joint; kinesis, as a result, is limited. No orbitosphenoid present; septum ossifies as mesethmoid which appears on the dorsal surface. There are only two circumorbital bones present: the lacrimal and the jugal. The auditory region has only two centres of ossification: the prootic and opisthotic. The quadrate has a single elongated condyle of the processus oticus which articulates with the prootic and squamosal. The cranial base is ossified as the

basioccipital and basisphenoid, the latter being of mixed origin. There are five dermal bones in the lower jaw of *Struthio*; the gonial is present. The trigeminus musculature is reduced and shows very definite neotenic features. The peripheral cranial nerves are typically avian. The cranial parasympathetic nerves are well developed in the embryo but show definite signs of resorption in the later stages of development. The hyoid apparatus is mainly cartilaginous; the hyoid musculature is reduced.

I. HISTORICAL INTRODUCTION

De Villiers (1946) and McDowell (1948) have both gone into considerable detail concerning the historical aspect of the so-called "*Ratitae*". It is, however, advisable to combine these accounts, thus bringing the existing knowledge up to date.

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THE CLASSIFICATION OF THE "PALAEOGNATHAE"

There has been no specific work on the osteocranium of *Struthio* since W. K. Parker's paper on the structure and development of the skull in the ostrich tribe (1866). Since then Lowe (1928) discussed the phylogeny of the ostrich and its allies, and followed up this work with additional information on the phylogeny of the *Struthiones* in 1942—1943. Frank (1954) described the ontogenesis of the chondrocranium of *Struthio*. Apart from these, very little work has been done on the skull of *Struthio* as such.

There are, however, numerous papers on the phylogeny of the *Ratitae* or so-called walking birds in general. Among these the most important early works are those of W. K. Parker (1866 a) on the gallinaceous birds and tinamous, T. J. Parker (1891) on the anatomy and development of *Apteryx* and (1895) on the cranical osteology, classification and phylogeny of the *Dinornithidae*.

These works have formed the basis for all subsequent studies of the *Ratitae* and have since been followed by that of Kesteven (1942) on the ossification of the avian chondrocranium with special reference to that of the emu. De Villiers (1946) investigated the palate of the tinamou (*Crypturellus*).

Merrem (1816) coined the word *Ratitae* to include *Struthio, Rhea, Dromiceius* and *Casuarius*. *Apteryx* was not included and the tinamous were placed with the *Carinatae*. Huxley (1867) included *Apteryx* with the other genera mentioned by Merrem in a superorder *Ratitae*, still placing the tinamous with the *Carinatae*. Parker, Newton Andrews and Milne-Edwards were of the same opinion as Huxley, but also included the *Dinornithidae* and *Aepyornithidae* in the dromaeognathic group. These fossil forms were possibly unknown to Huxley (McDowell, 1948).

Huxley (1867) coined the word "*Dromaeognathae*" to include those birds which conform to a definition based upon the structure of the palate (see next

paragraph). PYCRAFT (1900) replaced "dromaeognathic" with "palaeognathic", but at the same time included *Crypturi* with Huxley's "dromaeognathic" forms and defined the *Palaeognathae* as differing from all other known birds "... in that, in the adult, the pterygo-palatine connection is by symphysis or ankylosis, and not by arthrosis." (PYCRAFT, 1900).

MCDOWELL (1948) considers that the *Palaeognathae* is not a definable group, because in Huxley's definition of the palaeognathic condition the posterior extremity of the vomer is said to be produced backwards to receive the anterior extremity of the pterygoid and the posterior extremity of the palatine, the vomer thus preventing the pterygoids and palatines from reaching the parasphenoidal rostrum. MCDOWELL (1948) points out that this definition is wholly adequate for the *Tinamidae* and *Rhea* and not for the remainder of the group, and for the following reasons:

1) BEDDARD (1898) pointed out that *Struthio* must be excluded, because while the vomer and pterygoid do not meet, the latter does meet the parasphenoid. This is also true for *Apteryx*.

2) MCDOWELL (1948) pointed out that in some *Neognathae*, such as *Anhima* and the *Anseres*, the pterygoids and vomers are narrowly separated. Moreover, he mentions that in many *Neognathae* the palate is suspended beneath the parasphenoidal rostrum, so that neither the pterygoid nor palatine touches it, a condition obtaining in the fowl and duck as well as in *Casuarius* and *Dromiceius*. Huxley's definition of the palaeognathic palate requires the vomer to be large, but according to PYCRAFT (1900) and MCDOWELL (1948) it is small and reduced in *Struthio* in comparison with the other *Dromaeognathae*; and *Diomedea* of the *Neognathae* has a large vomer (MCDOWELL, 1948). The last point in Huxley's definition is the backward position of the basipterygoid process, and this can be matched by the *Musophagidae* and *Turnidae* of the *Neognathae* (MCDOWELL, 1948).

McDowell's criticism of Pycraft's definition is that *Dromiceius* does not have a palato-pterygoid arthrosis, and that numerous *Neognathae* have a squamous sutural articulation between palatine and pterygoid. For these reasons MCDOWELL (1948) considers that the palaeognathous palate is no true morphological reality. Thus MCDOWELL (1948) on morphological grounds alone, considers that the *Palaeognathae* cannot be defined, and that they fall into four main groups; but through lack of additional evidence the affinities of these groups to one another and to the *Neognathae* are not known. He is also not prepared to state definitely that they are a reversal from a neognathous stock or stocks, or that they are truly primitive. As a result of the uncertainty of the group morphologically and ontogenetically, he suggests that the superorder *Palaeognathea* be dropped from the classification of birds, and that the orders previously placed in this superorder be included within the *Neornithes*, thus not separating them from the birds hitherto known as the "*Neognathae*".

II. MATERIAL AND TECHNIQUE

For a full account of the material used and the technique employed the reader is referred to Frank's work on "The Development of the Chondrocranium of the Ostrich". The material prepared by him was used in this investigation, together with three other stages—17, 27 and 37 day old embryos. These had also originally been collected by FRANK (1954) and were fixed and preserved in Allen's fluid P.F.A.3 (MCCLUNG, 1937). This fixative is ideal in that the material can be left in it for an unlimited period without the danger of over-fixing; and in that at the same time it acts as a decalcifying agent.

The embryos, after having been in Allen's fluid for approximately two years, were transferred directly to 70 % Alcohol without previously washing in water. BRAUER (1949) maintains that all material treated with a formol fixative must not be washed in water so as to prevent swelling. The picric acid can, if desired, be removed by adding a trace of lithium carbonate to the alcohol. It was, however, found unnecessary to decolourize the material as the presence of the yellow colour in no way affected the end results.

The embryos were placed in three changes of alcohol over a period of three days, and then transferred to the bulk-stain, borax carmine. After embedding, they were sectioned at 20 μ and counterstained in azan. The results obtained were excellent giving exceedingly clear differentiation of the structures and tissues.

Graphic and contour reconstructions were made according to the method described by PUSEY (1939).

Apart from the embryonic material used in this investigation, adult material, obtained from Oudtshoorn, was also used. Thirty-six heads in all were collected at random and were despatched by aircraft, having previously been packed in sealed tins to which had been added liberal quantities of thymol crystals to prevent putrefaction. On arrival at the institute the heads were stored in a refrigerator in which were placed thymol crystals. It was found that they remained fresh for a considerable time, thus making it possible for the macroscopical study to be extended over a period of a month without having to preserve them in formalin.

For comparative purposes, *Rhea americana* and *Crypturellus Sp.* were used, the former having been sectioned by Frank and the latter by Professor C. G. S. de Villiers.

III. ACKNOWLEDGMENTS

I wish to record my very sincere thanks to Professor C. G. S. de Villiers, who, at all times showed considerable interest in this investigation, and for his

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THE STRUCTURE OF THE ADULT PALATE AND ITS VARIATIONS

A special study of thirty adult heads was made in an endeavour to determine the relations of the bones constituting the roof of the oral cavity.

Considerable attention was paid to the vomer, not only to its specific measurements, but also to its relation to the pterygoid, as this is an important factor in distinguishing the *Dromaeognathae* (HUXLEY, 1867) or *Palaeognathae* (PYCRAFT, 1900) from the *Neognathae* (PYCRAFT, 1900).

The bones constituting the oral roof are the paired maxillaries, palatines and pterygoids, and the vomer and premaxillaries which are unpaired in the adult. All these bones, excluding the vomer, are relatively constant in shape and size and conform to the basic plan recognized for the ostrich where the palato-pterygo-quadrate bar is the straightest of any bird and runs parallel to

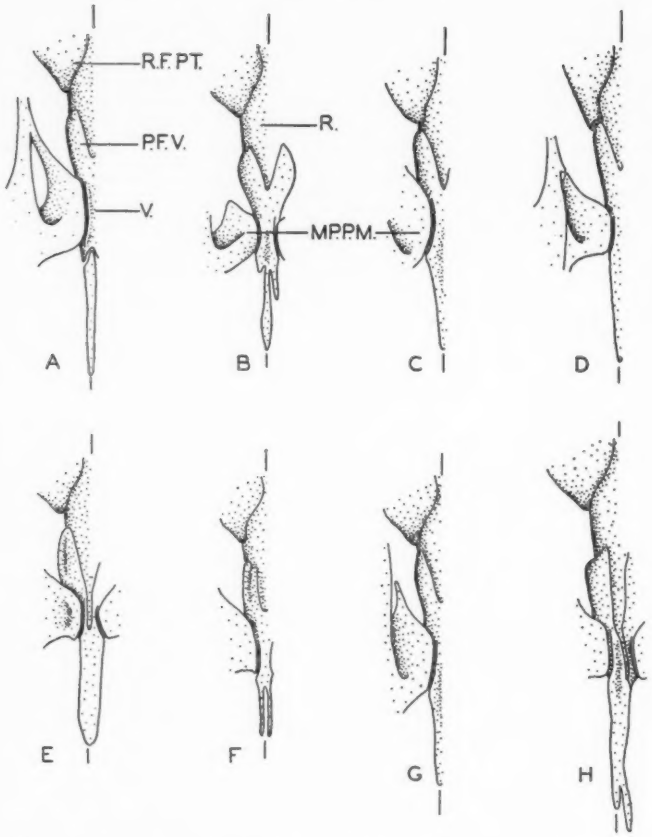

Fig. 1. Sketches of the palate in the region of the vomer, pterygoid and maxillary in a number of adults (½ natural size). *M.P.P.M.*, maxillo-palatine process of maxillary; *P.F.V.*, posterior flange of vomer; *R.*, rostrum; *R.F.P.T.*, rostral flange of pterygoid; *V.*, vomer.

the rostrum (DE VILLIERS, 1946). The vomer is the element variable in size and in its relations to the neighbouring bones of the primary and secondary palate.

The most significant feature of the heads studied is the uniform cranial length: the heads were collected at random. It was found that the vomerine length and the distance between the vomer and the antero-medial process of the rostral flange of the pterygoid bear no constant relation to each other. Also, it was found that the vomerine length was in no way proportional to the cranial length.

The greater the vomerine length the greater is the chance that the vomer will lie nearer the pterygoid. Because of the length of the vomer, it need not neces-

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sarily follow that it will lie close to the pterygoid. In fact, in only three of the heads studied in this investigation, does the vomer push against the pterygoid. However, it was found that a shorter vomer does not increase the vomero-pterygoid distance.

It is clear that though no hard and fast rule can be applied to the vomerine and cranial lengths and the vomeropterygoid distance, it can be said, though tentatively, that the distance between the vomer and pterygoid is dependent on the length of the vomer, but there are exceptions, and were a greater number of heads studied this assumption might be proved false.

The vomer anteriorly is undivided (Fig. 1) in all but a few cases, and from the arthrosis with the maxillopalatine process it tapers in most cases to a point, while in a few instances it is broad and terminates bluntly (Fig. 1). In only one of the specimes studied was the vomer cleft anteriorly (Fig. 1 F). In two of the thirty heads studied the vomer is cleft between the maxillo-palatine processes; in one the cleft is median and in the other asymmetrical (Fig. 1 E and H).

In about a quarter of the heads studied it was found that the anterior tip of the vomer was separated from the main body of the element (Fig. 1 A), and formed what might be considered as a separate bony structure. Since this is an exception it must be considered as an incomplete ossification or as a fracturing of the vomer anteriorly, thus giving the appearance of a separate osseous element or prevomer. Anterior to the arthrosis between the maxillo-palatine process and the vomer, the vomer is thin and flattened as well as being pneumatic, thus fracturing could easily take place. In none of the ten embryos studied does there appear a separate ossification centre anterior to the vomer.

The anterior part of the vomer, up to its arthrosis with the maxillo-palatine process, is spatulate and loosely connected to the overlying rostrum. Beyond the arthrosis it is trough-shaped, closely sheathing the rostrum. Its hindermost part is interrupted medially by a posterior incision.

The portion of the vomer lying between the maxillo-palatine processes is slightly trough-shaped ventrally, this condition being brought about by the flattening of the lateral surfaces of the vomer in this region, to accomodate the maxillary processes, thereby forming a syndesmosis (Fig. 1).

Anteriorly the fused *premaxillaries* form the anterior and antero-lateral margins of the palate. It displays a triradiate pattern with a median and two lateral processes extending backwards. These processes arise from the body of the premaxillary which is dorso-ventrally flattened, being slightly concave ventrally. The two lateral processes—palatal processes of the premaxillaries—constitute the antero-lateral elements of the palate. They are broad where they leave the body of the premaxillary but rapidly taper into splint-like bones, which extend posteriorly lateral to the maxillary and may reach as far back as the jugal. In either case the union between the premaxillary and maxillary

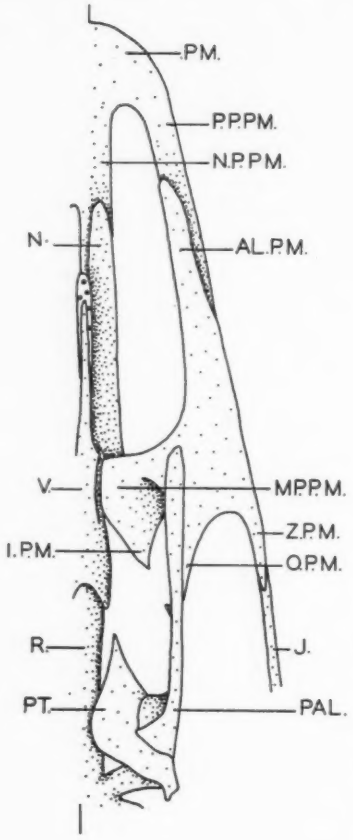

Fig. 2. Sketch of the adult palate. *AL.P.M.*, antero-lateral process of maxillary; *I.P.M.*, inner process of maxillary; *J.* jugal; *MP.P.M.*, maxillo-palatine process of maxillary; *N.*, nasal; *N.P.P.M.*, nasal process of premaxillary; *O.P.M.*, outer process of maxillary; *PAL.*, palatine; *PM.* premaxillary; *P.P.P.M*, palatal process of premaxillary; *PT.*, pterygoid; *R.*, rostrum; *V.*, vomer; *Z.P.M.*, zygomatic process of maxillary.

or the premaxillary and jugal, respectively, is rigid without the elements being synostotically connected. The median unpaired process of the premaxillary—the nasal process—extends backwards dorsal to the nasals (Fig. 2).

The body of the maxillary is dorso-ventrally flattened and forms the major portion of the bony palate. It is produced into five distinct processes (Fig. 2), three of which are posteriorly directed.

(a) The antero-lateral process of the maxillary which is dorso-ventrally flattened lies medio-ventrally to the palatal process of the premaxillary forming with the latter the lateral border of the anterior palatal vacuity (Fig. 2).

(b) The zygomatic process of the maxillary extends backwards making contact with the jugal. The union so established (Fig. 3) is peculiar in that the process is split posteriorly into a dorsal and ventral portion, each of which tapers to a point lying above and below the jugal. Anteriorly the jugal is similar though vertically cleft, resulting in an inner and outer process, which interdigitate with the maxillary processes. The basic nature of the joint is

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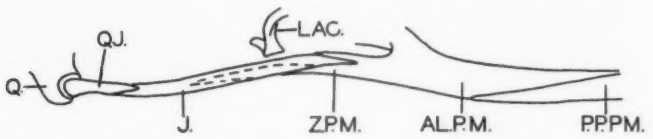

Fig. 3. Sketch of the lower temporal arch in the adult. *AL.P.M.*, antero-lateral process of maxillary; *J.*, jugal; *LAC.*, lacrimal; *P.P.P.M.*, palatal process of premaxillary; *Q.*, quadrate; *QJ.*, quadratojugal; *Z.P.M.*, zygomatic process of maxillary.

constant, but the length of either one or other, or both of the maxillary processes is variable. They may both be short, or there can be considerable asymmetry, the ventral splint being considerably elongated to reach the quadratojugal. In none of the heads studied does the dorsal process extend beyond the region of the lacrimal.

(c) The outer process of the maxillary (Fig. 2) extends backwards and is closely applied to the palatine. It is dorso-ventrally flattened and relatively broad proximally, but soon tapers to a thin, laterally compressed process on the side of the palatine.

(d) The maxillo-palatine process of the maxillary forms a broad "foot-plate" to the body of the maxillary and develops an arthrosis with the vomer (Fig. 2).

(e) The inner process of the maxillary is of considerable importance because of its relation to the palatine. It is in all cases connected to the palatine by a ligament, but the space between the process and the palatine is variable; in some instances, even though the ligament persists, this process and the palatine are in synostosis (Fig. 1 A and 1 D). In size and shape the process is variable being either short and blunt or long and tapering, or showing asymmetry on the left and right hand sides.

The *Palatine* is rod shaped and lies parallel to the rostrum. The anterior end is narrow and dorso-ventrally flattened, and is not in synostotic continuity with the maxillary. Posteriorly it abuts against the pterygoid and may be in synostosis with it. Half way along its length, there is a small dorso-medially directed process (Fig. 2) which affords attachment for the ligament passing from the maxillary to the palatine (Plate 1).

The *Pterygoid* (Fig. 2), like the other bones constituting the palate, is dorso-ventrally flattened. The medial portion of the pterygoid overlaps the rostrum posteriorly and is known as the rostral flange of the pterygoid. Postero-laterally there is an arthrosis with the processus basipterygoideus. The posterior extremity of the pterygoid forms an arthrosis with the quadrate. There is a single large articulatory surface situated postero-laterally forming an arthrosis with the ventro-proximo-medial surface of the processus orbitoquadratus of the quadrate. Lastly, there is an anterior process of the pterygoid which is connected to the posterior flange of the vomer by a strong ligament. It is unfortunate that those

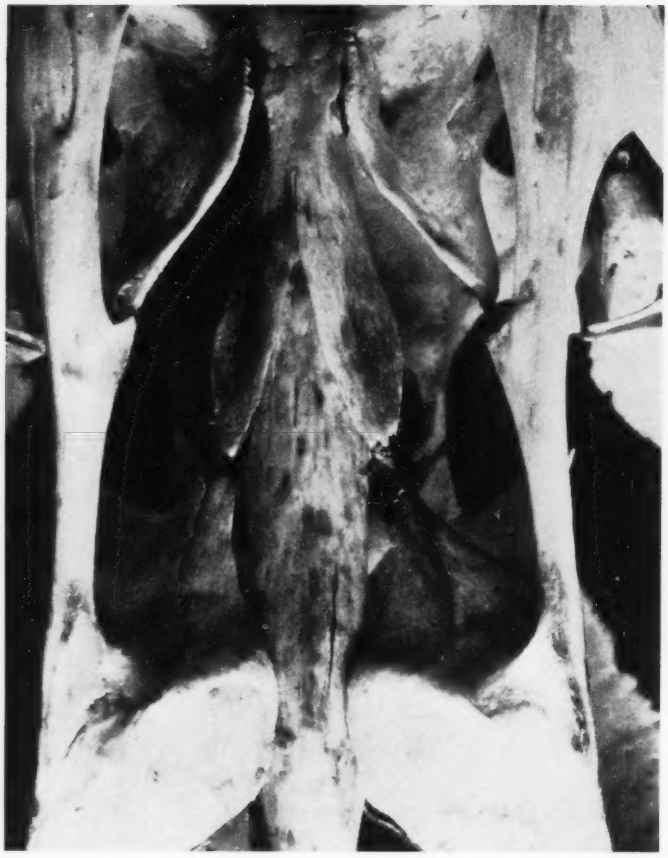

Plate 1. Photograph of the ventral aspect of the skull of the adult to illustrate the relation of the vomer and pterygoid, and palatine and maxillary.

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specimens in which the vomer reaches the pterygoid were not preserved immediately so that the histological details could be determined.

THE ONTOGENY, ARTHROSES AND RELATIONS OF THE BONES OF THE PALATE

A. ONTOGENY

All the bones constituting the osseous palate are dermal in origin, and each arises from areas of mesenchyme condensations in which are differentiated the osteoblasts which give rise to the bony elements by the deposition of collagen fibrillae. Those osteoblasts that are already enclosed in the collagen

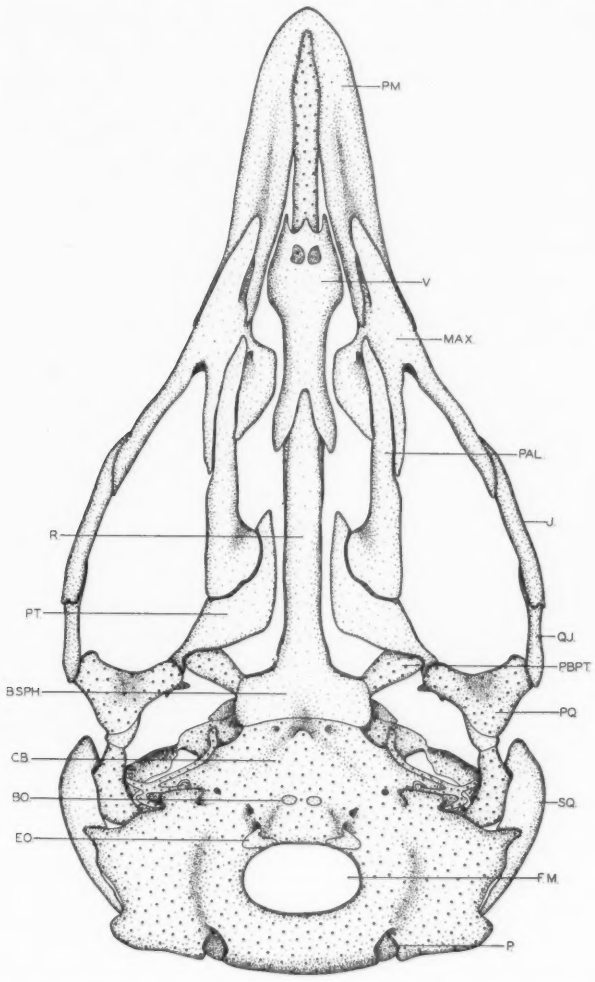

Fig. 4. Graphic reconstruction of the skull of the 21-day old embryo × 5. Ventral aspect. *BO.*, basioccipital; *BSPH.*, basiphenoid; *C.B.*, cranial base; *EO.*, exoccipital; *F.M.*, foramen magnum; *J.*, jugal; *MAX.*, maxillary; *P.*, parietal; *PAL.*, palatine; *P.BPT.*, processus basipterygoideus; *PM.*, premaxillary; *P.Q.*, pars quadrata; *PT.*, pterygoid; *QJ.*, quadratojugal; *R.*, rostrum; *SQ.*, squamosal; *V.*, vomer.

are called osteocytes, and the periphery of the formative area is beset with a definite zone of osteoblasts that will gradually increase the size of the bone by appositional growth.

The palatal bones all appear at more or less the same stage of development. In the 14-day stage, though there are condensations of mesenchyme and osteoblasts, no collagen has yet been deposited. In the 15-days stage, the bones

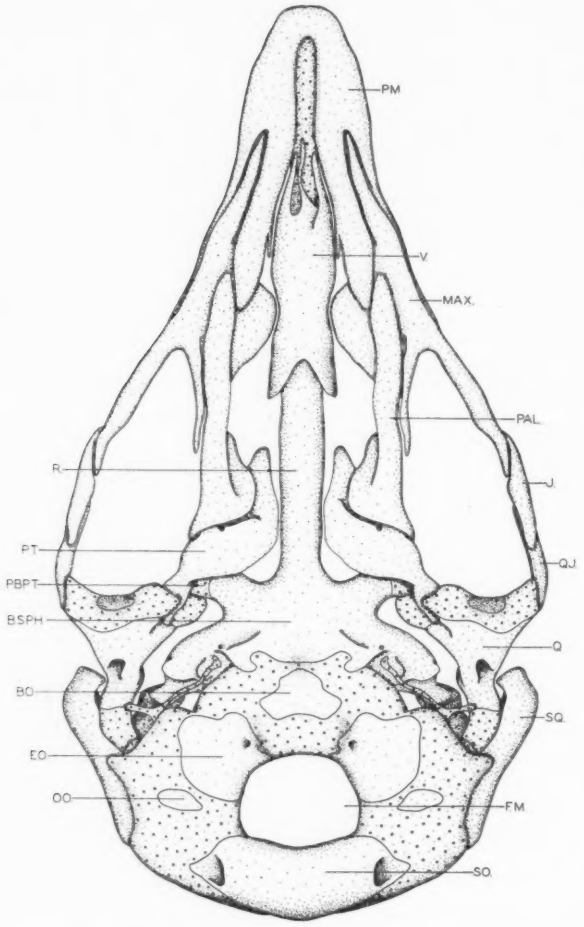

Fig. 5. Graphic reconstruction of the skull of the 34-day old embryo. × 2·5. Ventral aspect. *BO.*, basioccipital; *BSPH.*, basisphenoid; *EO.*, exoccipital; *F.M.*, foramen magnum; *J.*, jugal; *MAX.*, maxillary; *OO.*, opisthotic; *PAL.*, palatine; *P.BPT.*, processus basipterygoideus; *PM.*, premaxillary; *PT.*, pterygoid; *Q.*, quadrate; *QJ.*, quadratojugal; *R.*, rostrum; *SO.*, supraoccipital; *SQ.*, squamosal; *V.*, vomer.

as such are recognizable though they are very weakly formed and widely spaced; in subsequent stages they increase in length and breadth by appositional growth, and so make contact with each other.

All the bones constituting the palate arise as paired structures except the premaxillary and vomer. The very earliest discernible anlagen of these two bones are unpaired. This condition is undoubtedly secondary as both these bones are fundamentally paired structures. The premaxillary forms a cap over the prenasal process and spreads backwards as three distinct processes, one

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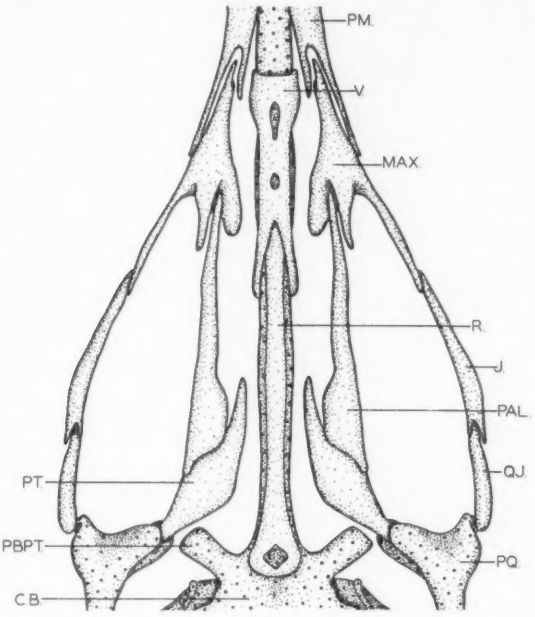

Fig. 6. Graphic reconstruction of the palate in the 19-day old embryo. × 6·7. Ventral aspect. *C.B.*, cranial base; *J.*, jugal; *MAX.*, maxillary; *PAL.*, palatine; *P.BPT.*, processus basipterygoideus; *PM.*, premaxillary; *PQ.*, pars quadrata; *PT.*, pterygoid; *QJ.*, quadratojugal; *R.*, rostrum; *V.*, vomer.

dorso-medial and two lateral. The two lateral processes make contact with the maxillary posteriorly.

The vomer is a median unpaired element lying immediately ventral to the rostrum. Early in ontogeny the posterior division of the vomer does not show the characteristic trough-shaped appearance which it shows later in development. Anteriorly the bone is cleft with two lateral processes, and in later stages (Figs. 4 and 5) it possesses three processes: two lateral, and one median. In the 21-day stage (Fig. 4) there are two foramina on either side of the middle line, while in the 19-day stage (Fig. 6) there are two foramina, one behind the other in the middle line. This may be important as indicating the remnants of a paired vomer in reptiles. Posteriorly the vomer has the characteristic lateral flanges on either side of the rostrum and separated by a V-shaped cleft, (Figs. 4, 5 and 6) perhaps itself indicative of the original paired nature of the vomer.

The maxillaries, palatines and pterygoids are deposited where they will be found in the late stages of ontogeny or the adult, though their relations one to another are not yet established (Fig. 6).

The parasphenoid will be described in the section on the ontogeny of the ossa substitientia.

B. ARTHROSES AND PALATAL OSSEOUS RELATIONS

There are three types of arthroses involved in the palate, namely synovial, sliding syndesmosis and fixed immovable syndesmosis.

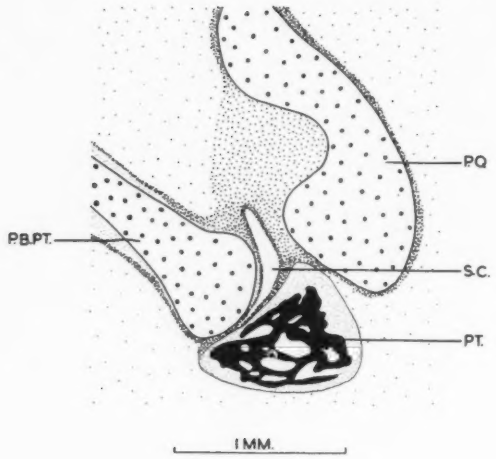

Fig. 7(a).

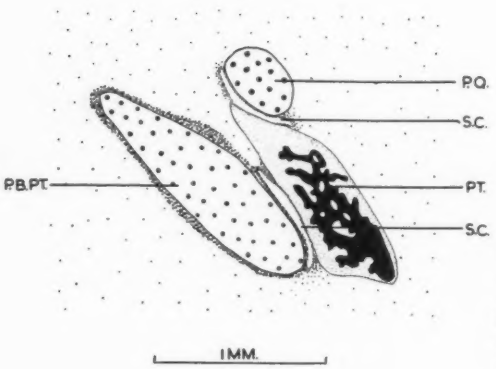

Fig. 7(b).

Fig. 7. Transverse section illustrating the synovial arthrosis between the processus basipterygoideus and pterygoid. a) In the 27-day old embryo. b) In the 21-day old embryo. *P.BPT.*, processus basipterygoideus; *P.Q.*, pars quadrata; *PT.*, pterygoid; *S.C.*, synovial cavity.

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(1) Synovial Joints

There are two such joints: the one between the pterygoid and processus basipterygoideus, and the other between the pterygoid and quadrate bone (Fig. 7 A and 7 B). In the former the posterior surface of the pterygoid is grooved to receive the distal end of the processus basipterygoideus (Figs. 4 and 5) which is cartilaginous, while the surface of the pterygoid involved in the joint does not possess a cartilaginous cap. The joint between the pterygoid and the quadrate is quite extensive but neither of the surfaces have cartilage at the point of articulation (Fig. 33). This is also the case in the adult.

In the development of the two joints, which appear relatively late in ontogeny, the space between the bones involved is filled with what HAM (1950) calls an

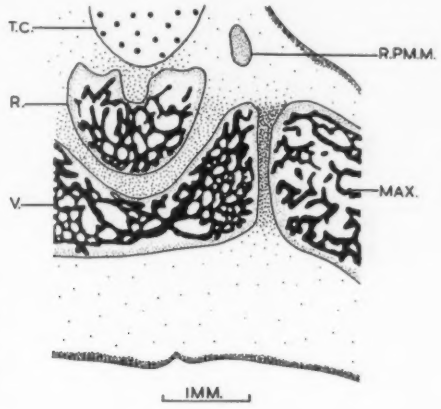

Fig. 8(a). Transverse section of the vomer-rostral arthrosis, and the vomer maxillary arthrosis in the 34-day old embryo. *MAX.*, maxillary; *R.*, rostrum; *R.P.M.M.*, ramus premaxillaris medialis; *T.C.*, trabeculae communis; *V.*, vomer.

articular disk of mesenchyme or primitive joint plate which is continuous with the perichondrium and/or periosteum. With the progress of development the mesenchyme disk becomes areolar in nature. The neighbouring spaces coalesce giving rise to a continuous space, so forming the synovial cavity, and permitting of movement between the two bones involved. The cavity is then bounded by mesenchyme which is continuous with the periosteum or perichondrium as the case may be, and it becomes differentiated into two distinct layers: an outer fibrous layer and an inner thin synovial membrane. The former layer is continuous with the fibrous layer of the perichondrium or periosteum.

(2) Sliding Syndesmosis

This type of connexion is found only between the vomer and rostrum. In the region of the arthrosis with the maxillary the vomer is trough-shaped dorsally (Fig. 8 A) covering the ventral surface of the rostrum upon which it is able to move. The connective tissue holding the vomer in position against the rostrum is loose, thus permitting limited movement.

(3) Immovable Syndesmosis

All other inter-relations of neighbouring bones of the palate can be classified under this heading. The pterygo-palatine connexion is the simplest of all in as much as the palatine lies ventral and lateral to the rostral flange of the pterygoid, fitting into a slight excavation of the surface of the pterygoid (Figs. 4 and 5), thus bringing the surfaces of the two bones to the same level. The periosteum in the early stages of ontogeny becomes differentiated into an outer fibrous layer which later becomes thickened and forms a tough sheath investing both bones and an inner layer of osteoblasts giving rise to the collagen material of the growing bone (Fig. 9).

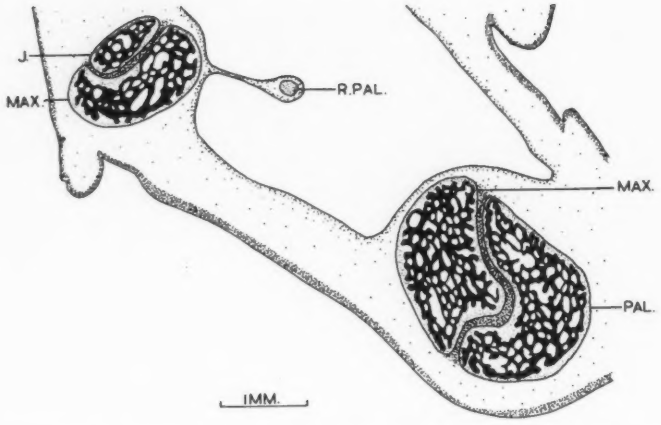

Fig. 8(b). Transverse section, illustrating the maxillo-jugal and maxillo-palatine arthroses in the 34-day old embryo. *J.*, jugal; *MAX.*, maxillary; *PAL.*, palatine; *R.PAL.*, ramus palatinus.

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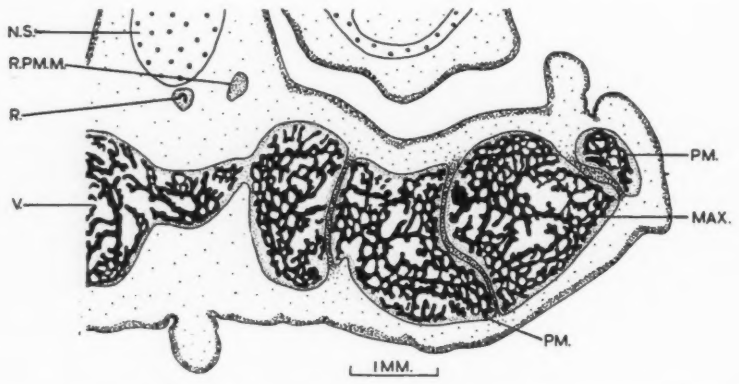

Fig. 8(c). Transverse section of the vomer-premaxillary and premaxillary-maxillary arthroses in the 34-day old embryo. *MAX.*, maxillary; *N.S.*, nasal septum; *PM.*, premaxillary; *R.*, rostrum; *R.PM.M.*, ramus premaxillaris medialis; *V.*, vomer.

The connexion between the palatine and maxillary is such that the surface of the former has been hollowed out so as to receive the maxillary, resulting in a flush surface. The length of the connexion is quite extensive and incorporates approximately half the palatine (Figs. 4 and 5). Both these bones are enclosed in a fibrous sheath derived from the periosteum; lying between the two elements is a thin layer of connective tissue (Fig. 8 B).

The connexion between the maxillary and premaxillary is extremely stable. The palatal process of the premaxillary and the antero-lateral process of the maxillary are so constructed as to interdigitate (Figs. 4 and 5).

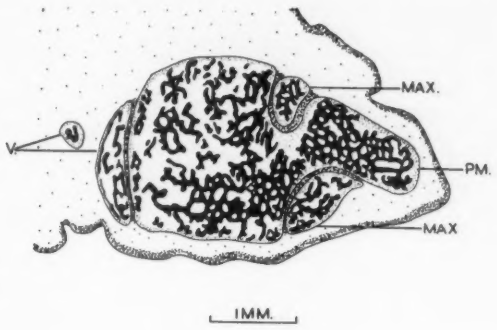

Fig. 8(d). Same as 8(c) but further anterior. *MAX.*, maxillary; *PM.*, premaxillary; *V.*, vomer.

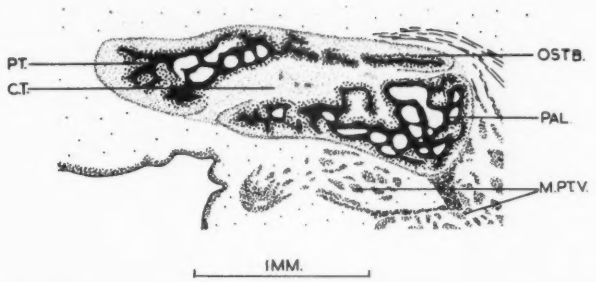

Fig. 9. Transverse section, illustrating the pterygo-palatine arthrosis in the 27-day old embryo. *CT.*, connective tissue; *M.PT.V.*, m.pterygoideus ventralis; *OSTB.*, osteoblasts; *PAL.*, palatine; *PT.*, pterygoid.

The histological nature of this connexion is the same as that between the maxillary and palatine, and palatine and pterygoid (Figs. 8 b and 9).

Finally, there is the connexion between the maxillo-palatine process of the maxillary and the vomer. The arthrosis is established late in ontogeny (cf. Figs. 4, 5 and 6). The opposed surfaces are flattened and between them is a thin disk of connective tissue, the two bones being held together by a common fibrous capsule derived from the periosteum.

A condition in the ontogeny and persisting late, but not reflected in the adult, is a connexion between the vomer and the palatal processes of the premaxillaries (Figs. 4 and 5). This joint is purely embryonic as, of the thirty heads studied not one has the vomer and premaxillary approaching each other as in the embryo where there is a double connexion of the vomer with the premaxillary and maxillary. Only the latter connexion is retained in the adult. The histological nature of this connexion is identical with that of the vomero-maxillary joint (Fig. 8 C and D).

Discussion

PARKER (1866) dealt with the palatal bones rather sketchily, and consequently, the interpretation of his work becomes very difficult. There are, however, some interesting features to be commented upon.

The vomer is an "azygous" element which is not generally cleft anteriorly, but according to PARKER (1866) it seems to be the case. Posteriorly it is cleft. PARKER (1866) refers to the "intermaxillary splints" which are none other than the palatal processes of the premaxillary which connect with the maxillaries, but actually arise from the main body of the premaxillary, which itself is unpaired, though this unpaired condition is secondary. Where the premaxillary and maxillary interdigitate, the premaxillary is divided into two distinct processes, median and lateral, with the median one touching the vomer. It is this process that PARKER (1866) refers to as the "prevomer"; it does touch the vomer but only in the embryo, and is at no time during ontogeny a separate bone.

In all the ratite birds investigated by PARKER (1866) and in the tinamou also investigated by PARKER (1886 a), the medial premaxillary splint connected to the vomer is referred to as the "prevomer". In *Apteryx*, PARKER (1891) finds no "prevomer", though there is the typical triradiate premaxillary. Parker's "prevomer" has, of course, nothing to do with the prevomer of BROOM and BROCK (1931), DE BEER (1937) and others. BROOM and BROCK (1931) consider the bone commonly called vomer in birds a prevomer. The whole problem of the "prevomer" of Broom and others has now been proved to be purely academic. The arguments for and against are reviewed by PARRINGTON and WESTOLL (1940).

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THE ONTOGENY, ARTHROSES, AND RELATIONS OF THE DERMAL ROOFING BONES OF THE SKULL

(1) ONTOGENY

The roofing bones are all laid down in the same manner as has been described for the palatal bones. The anterior elements are the first to appear, with those forming the cranial roof developing slightly later.

There is only one median unpaired element: the nasal process of the premaxillary. This is, of course, secondary as the premaxillary is fundamentally a paired structure. The nasal process extends back to reach the nasals, which are paired elements. Both the nasals and the premaxillary are well developed in the 21-day stage, in which the frontals are very weakly developed and form

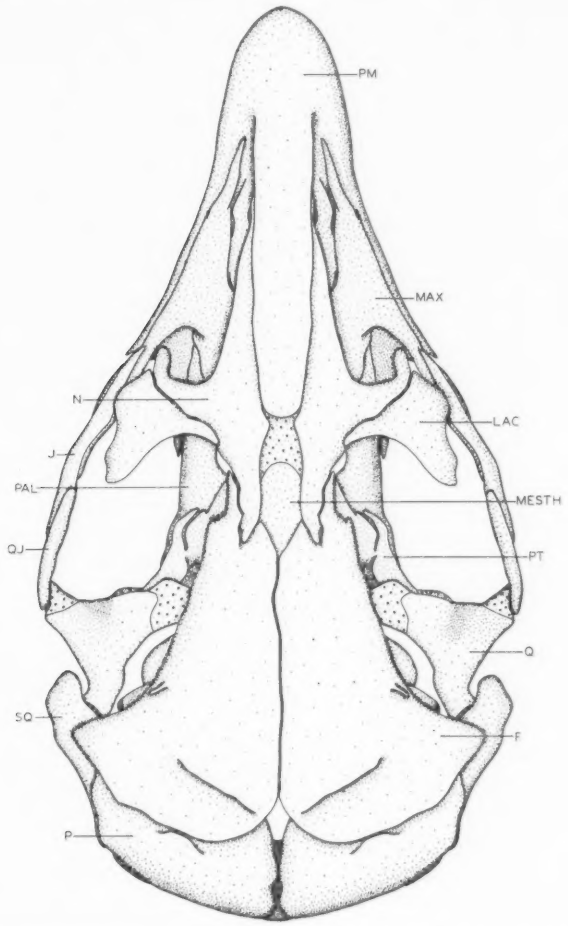

Fig. 10. Graphic reconstruction of the skull of the 34-day old embryo. × 2·5. Dorsal aspect. *F.*, frontal; *J.*, jugal; *LAC.*, lacrimal; *MAX.*, maxillary; *MESTH.*, mesethmoid; *N.*, nasal; *P.*, parietal; *PAL.*, palatine; *PM.*, premaxillary; *PT.*, pterygoid; *Q.*, quadrate; *QJ.*, quadratojugal; *SQ.*, squamosal.

the lateral margins of the cranial vault leaving the dorsal part of the vault uncovered. Posteriorly the parietals are present but do not meet the frontals or each other in the middle. Posteriorly they overlie the tectum synoticum.

By the 27-day stage, the nasals and the premaxillary have increased in length and thickness, and their relation to each other is more intimate, though the two nasals do not form a median dorsal suture. The frontals have undergone considerable development and now meet in front forming a short suture. The parietals likewise are suturally connected in the region of the tectum synoticum, but anteriorly are widely separated. The posterior margin of the frontals

underlies the parietals so forming the coronal suture. Dorsally, therefore, there is a large diamond shaped area still unossified.

By the 34-day stage the roofing bones are more or less similar to those of the adult. The frontals and parietals close the cranial vault except for a small unossified area (Fig. 10). The coronal, sagittal and lambdoidal sutures are well developed.

The Adult

Most of the sutures are persistent in the adult but there seems to be a tendency, perhaps in older individuals, for the medial frontal, the sagittal and the lamdboidal sutures to be lost through synostosis, thus forming a rigid cranial roof.

The nasal process of the premaxillary extends backwards, and its distal extremity tapers and comes to lie between the nasals and forms the anterior border of a temporary anterior fontanelle. In all but a few cases the distal extremity is cleft, with the right and left processes lying lateral to the fontanelle.

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The nasal is characteristically triradiate and forms the posterior and medial borders of the preorbital fossa. The anterior processes of the nasals which are in sutural continuity with each other, are overlaid anteriorly by the nasal process of the premaxillary. The posterior processes of the nasals form the lateral borders of the anterior fontanelle. Finally, there is the latero-ventral process which ends a short distance above the zygomatic process of the maxillary. In none of the heads studied do they meet.

The frontals are the largest bones of the skull and cover the whole of the dorsal orbital region and the anterior part of the cranial vault. Anteriorly the orbital ledge of each frontal extends forwards as two processes and in a few cases these join with the lacrimal thus forming a fontanelle bounded medially and laterally by the frontal and anteriorly by the lacrimal.

Laterally the fontal has a ventrally directed flange which joins the "orbitosphenoid" and pleurosphenoid, thus forming the dorsal and part of the posterior wall of the orbit. Posteriorly, the frontal is produced into a well pronounced process: the postorbital process.

The posterior roof of the brain case is formed by the parietals which are anteriorly suturally connected with the frontals forming with them the coronal suture. The temporary upper fontanelle is now completely closed. Posteriorly the parietals form the lambdoidal suture with the supraoccipital. Laterally the sutures always persist between the parietals and squamosal and pleurosphenoid.

The greater part of the brain case is enclosed laterally by the squamosal. It is a moderately small bone, and the sutures which it makes with the exoccipital and pleurosphenoid are not distinct in some of the adult heads. Extending latero-ventrally from the body of the bone is a pronounced zygomatic process, which

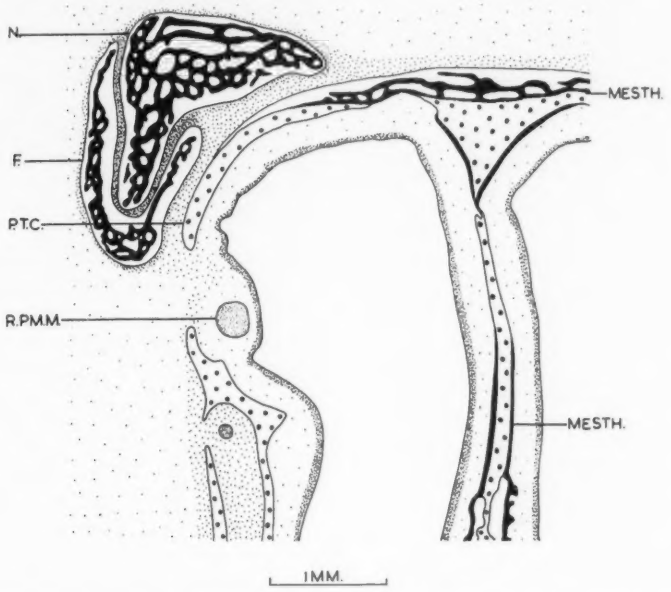

Fig. 11. Transverse section illustrating the nasal frontal arthrosis in the 37-day old embryo. *F.*, frontal; *MESTH.*, mesethmoid; *N.*, nasal; *P.T.C.*, parietotectal cartilage; *R.P.M.M.*, ramus premaxillaris medialis.

on its medial surface forms an attachment for the processus oticus of the quadrate. The medial surface of the squamosal becomes excavated for the articulation of the head of the quadrate.

(2) THE RELATIONS OF THE DERMAL ROOFING BONES

The nasal process of the premaxillary, for the greater part overlies the anteriorly directed processes of the nasals; these processes are ensheathed by fibrous connective tissue which is derived from the periosteum. The space between the two bones is filled with connective tissue which is limited to a thin plate not exceeding 20 μ in thickness. Ventrally, the nasal process of the premaxillary is recessed anteriorly to receive the anterior nasal processes, which are suturally connected, and lie close together.

The connexion between the nasals and frontals is syndesmotic and no movement is possible between these two elements. The posteriorly directed processes of the nasals form, with the antero-lateral processes of the frontals, an interlocking mechanism (Fig. 11). Both elements are encased in a fibrous capsule and are separated by a thin layer of connective tissue. The space separating these two bones, during ontogeny, is reduced by the process of appositional growth so characteristic of the growth of the dermal bones.

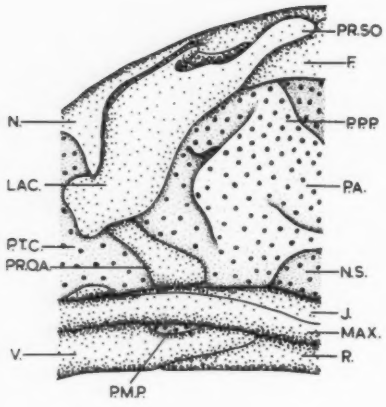

Fig. 12. Graphic reconstruction of the lacrimal in the 37-day old embryo. × 5. Lateral aspect. *F.*, frontal; *J.*, jugal; *LAC.*, lacrimal; *MAX.*, maxillary; *N.*, nasal; *N.S.*, nasal septum; *P.A.*, planum antorbitale; *P.M.P.*, processus maxillaris posterior; *P.P.P.*, post profundal process; *PR.O.A.*, processus orbitalis anterior; *PR.SO.*, processus supraorbitalis; *P.T.C.*, parietotectal cartilage; *R.*, rostrum; *V.*, vomer.

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The coronal suture does not permit any movement as the frontals and parietals are firmly united by connective tissue, and the posterior borders of the frontals overlie the parietals anteriorly. The lambdoidal suture is present in most cases but may be obliterated by synostosis of the parietals with the supraoccipital. The parietals actually rest on the dorsal surface of the occipital region.

The lacrimal is connected syndesmotically with the nasal and the frontal.

CIRCUMORBITAL BONES

A. THE LACRIMAL

The lacrimal is anteriorly situated in the facial region, and partly covers the anterior wall of the orbit, as well as forming the posterior margin of the foramen antorantorbitale which is confluent with the external nares. Dorsally the lacrimal is syndesmotically connected to the frontal posteriorly and the nasal antero-medially (Fig. 12). Projecting postero-ventrally from the main body of the bone is a prominent process which reaches down to the level of the jugal (Fig. 12 and Plate 2 A and B). Associated with this ventral process is a small bony element which fits into the grooved distal extremity of the process (Fig. 13 and Plate 2 A and B).

During ontogeny the lacrimal ossifies in the same way as the other membrane bones, and appears early in development about the 17th day of incubation. Apart from the jugal there is no other bone in the circumorbital region, which either develops separately, or later becomes fused with the lacrimal or frontal.

PYCRAFT (1900) mentions that in *Struthio* the lacrimal is extended backwards by means of fused supraorbital ossicles to join the frontal, which, as has been seen, is not actually the case. PARKER (1866) showed that there is only a lacrimal

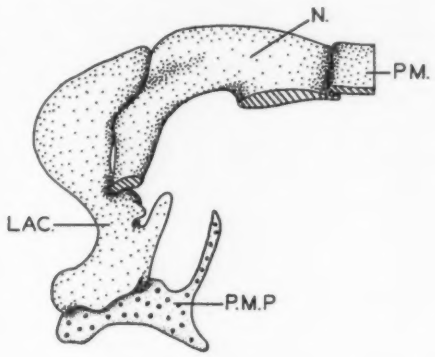

Fig. 13. Contour reconstruction of the lacrimal in the 37-day old embryo. × 5. Anterior view. *LAC.*, lacrimal; *N.*, nasal; *PM.*, premaxillary; *P.M.P.*, processus maxillaris posterior.

present which he figured in his drawings. He did, however, mention the presence of a prefrontal but this bone was not included in his drawings, and it can therefore be assumed that he was uncertain as to whether this bone should be termed the lacrimal or prefrontal.

STRESEMANN (1927—1934) and GOODRICH (1930) are noncommittal in their interpretation of the bone in question. The former, however, uses the term "präfrontale" and states that it is called by some authors the 'lacrimal". WIEDERSHEIM (1909) and BÜTSCHLI (1910) both use the term "Präfrontale" exclusively, while MARINELLI (1936) refers to this bone as the "lacrimal".

GAUPP (1902) homologises this bone with the lacrimal of mammals and considers that the reptilian element which lies medial to the ductus naso-lacrimalis should be termed the adlacrimal. GREGORY (1920) gave a comprehensive account of the prefrontal and lacrimal in different vertebrate classes. His account of the lacrimal (prefrontal) in birds is short and his conclusions are to a certain extent based on results of previous research workers, and he sums up the condition in birds as follows (1920, p. 129): "Nevertheless, in early stages of development, this bone, in *Struthio* and other birds, has more the appearance of a true reptilian prefrontal, as shown in figures by W. K. PARKER (1869), T. J. PARKER (1891) and PYCRAFT (1900). I, therefore, provisionally identify it as prefrontal rather than a lachrymal or as supraorbital." This deduction by Gregory is not convincing. GREWE (1951) considers that in *Anas* the topograhpical relation of the bone with membrane bones dorsal to it, the frontals and the nasals, is similar to the prefrontal of reptiles. He, however, considers that there are other factors that support the contention that this bone in birds is homologous to the lacrimal of mammals, or ventral bone of reptiles. The present author is of the same opinion that the bone anterior to the orbit is the lacrimal and not the prefrontal. GREGORY (1920, p. 128) rather supports this view when he states that: "In the whole class of birds the prefrontal is enlarged and simulates a mammalian lachrymal, since it transmits the lachrymal duct

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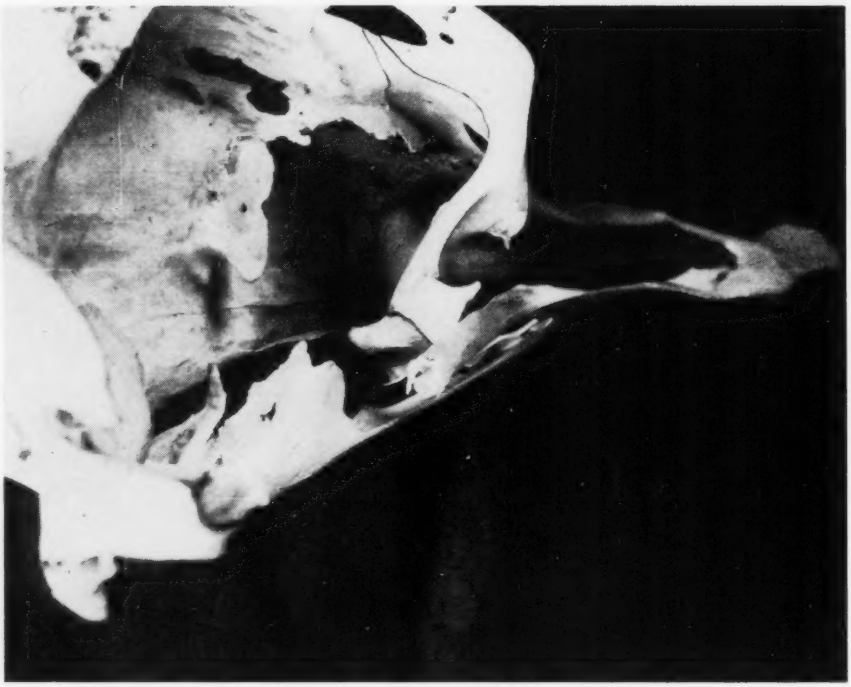
a



b

Plate 2. Photographs of the adult skull illustrating the lacrimal and its relation to the lower temporal arch. a) Lateral aspect. b) Postero-lateral view, also illustrating the uncinate process.

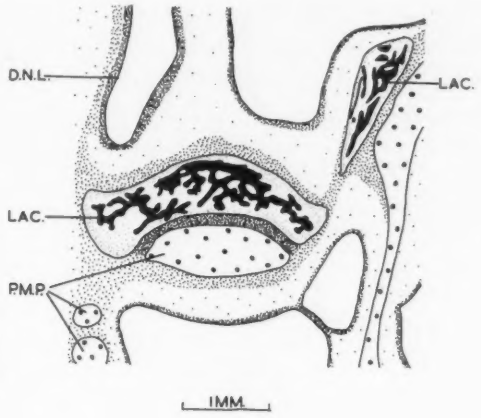


Fig. 14. Transverse section of the lacrimal-processus maxillaris posterior association in the 34-day old embryo. *D.N.L.*, ductus naso-lacrimalis; *LAC.*, lacrimal; *P.M.P.*, processus maxillaris posterior.

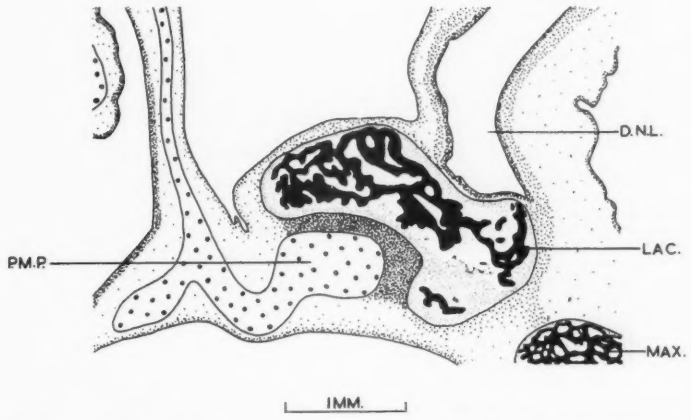


Fig. 15. Same as Fig. 14, but in the 37-day old embryo. *D.N.L.*, ductus naso-lacrimalis; *LAC.*, lacrimal; *MAX.*, maxillary; *P.M.P.*, processus maxillaris posterior.

and is located at the anterosuperior quarter of the orbit." Neither in *Anas* (GREWE, 1951) nor in *Nyctisyrigmus* (FOURIE, in press) is the lacrimal pierced by a formen; the ductus naso-lacrimalis lies lateral to the lacrimal in a groove. PYCRAFT (1900) maintains that in the *Palaeognathae* the lacrimal is pierced by a foramen. However, *Struthio* and *Rhea* both lack a foramen for the ductus naso-lacrimalis, while *Apteryx*, according to PARKER (1891), has a lacrimal because the antero-lateral element is pierced by a foramen. The position of the lacrimal in *Apteryx*, in relation to the nasals and frontals, is similar to that of *Struthio* and also of the other *Dromaeognathae* and "*Neognathae*". The characteristic peg-like process of the lacrimal reaches the lower temporal arch, and becomes loosely attached to the jugal in all the *Palaeognathae* PYCRAFT, 1900), and in a large number of *Neognathae*. This character is more typical of a

lacrimal than a prefontal (GREWE, 1951), even though, according to GREGORY (1920), the prefrontal in early *Amphibia* reached the jugal.

In *Struthio,* even though the lacrimal is not pierced by a foramen for the ductus naso-lacrimalis, the duct passes laterally to the ventral process and lies in a well pronounced groove. The duct is enclosed laterally by dense fibrous connective tissue which is continuous dorsally and ventrally with the periosteum of the lacrimal (Figs. 14 and 15).

STRESEMANN (1927—1934) considers the lacrimal (Präfrontale) as a protective bone which is highly pneumatic and is produced into strong processes in *Accipitres, Loridae, Turnicidae* and *Struthio.* The dorsal process is more or less horizontal and is referred to as the processus supraorbitalis, which is well developed in *Struthio* (STRESEMANN, 1927—1934 and MARINELLI, 1936) and also in the diurnal birds of prey (MARINELLI, 1936). There may also be a vertical process: the processus orbitalis anterior, which is the peg-like extension of the lacrimal (DE VILLIERS, 1946) and is present in *Sterna, Rhea, Anhinga* and *Phallacrocorax* (STRESEMANN, 1927—1934). In *Gallus* it is short, while in some forms it is long and curved beneath the orbit and may fuse with the processus postorbitalis or processus zygomaticus (MARINELLI, 1936).

Either the processus supraorbitalis or processus orbitalis anterior may be lengthened or broadened by the addition of separate bony plates. When these separate bony plates become fused with the processus orbitalis anterior, they are termed the ossa uncinata (BRANDT, 1840), and have been described for *Sterna* (SELENKA, 1891).

PARKER (1866) mentions the presence of a postfrontal in *Rhea,* the emu and tinamou. This is not confirmed by the author who was able to study serial sections of both *Rhea* and *Crypturellus* heads. But as both were adults it is possible that there might have been a separate bone in the embryo having subsequently become fused with the frontal. BÜTSCHLI (1910) states that the postfrontal is generally absent in birds.

MARINELLI (1936) describes the presence of bony plates that are present in the region of the frontal rim of the orbit, and reports that they are present in *Struthio, Accipitres, Psophia* and the tinamous. In the tinamous they lie adjacent to the frontal rim, while in the birds of prey they are free from the frontal rim.

B. THE JUGAL

The jugal forms the ventral boundary of the orbit and constitutes the major portion of the inferior temporal arch. The jugal, anteriorly, is rigidly connected to the zygomatic process of the maxillary (Fig. 8 B), and posteriorly with the quadratojugal. Both these connexions are similar, with the bones interdigitating. In *Struthio,* as in other birds, the temporal opening is confluent with the orbit because of the loss of both postfrontal and postorbital bones.

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THE ONTOGENY OF THE OSSA SUBSTITIENTIA (EXCLUDING THE OS QUADRATUM)

Up to the 27-day stage the chondrocranium shows few signs of ossification, while after this stage ossification proceeds rapidly, and by the 37-day stage, which was the last stage investigated, ossification is relatively far advanced. In the 37-day stage, however, cartilage still persists either between the encroaching ossification centres or between the two bony laminae formed during the initial stages of ossification.

The mode of bone formation of the chondrocranium is consistent throughout. The innermost layer of the perichondrium has the property of producing bone, so that the ossification of the chondrocranium is perichondral in nature. The enclosed cartilage is not ossified but is reduced and absorbed with the encroachment of bone. The ossification of the chondrocranium as a rule begins at the selvedges or margins, e.g. the margins of the foramen magnum or foramen prooticum; this is not consistent at there are ossification centres situated in the middle of a cartilaginous plate.

(a) Septum

The ossification of the septum begins late in embryonic development, being relatively well advanced by the 34th day, but is not present in the 27-day stage. In the 34-day stage the septum shows fairly advanced ossification by the formation of two perichondral laminae flanking a central plate of cartilage. The area of ossification is small, reaching neither to the trabecular beam ventrally nor to the cribriform plate dorsally. The position it occupies in the septum is neither wholly ethmoidal nor sphenoidal, but lies between these two regions, and can consequently be regarded as a combination of the vertical mesethmoid and orbitosphenoid.

The cribriform plate of the septum, that appears in the anterior fontanelle formed by the widely spaced nasals, shows slight ossification, which begins medially and spreads out laterally on the dorsal surface of the alae of the septum thus forming a dorso-medial mesethmoid. The ventral surfaces of the alae remain unossified till a later stage.

By the 37th day the vertical septum is further ossified by the peripheral addition of bony material to the centre of ossification. Ventrally the fused trabeculae show slight ossification by the presence of clusters of osteoblasts penetrating the substance of the cartilage, but are not derived from the interorbital centre of ossification. The trabeculae are separated from the vertical septum by the cartilage immediately above undergoing a change (Fig. 16). The cells become arranged in rows with their long axes horizontal; this layer

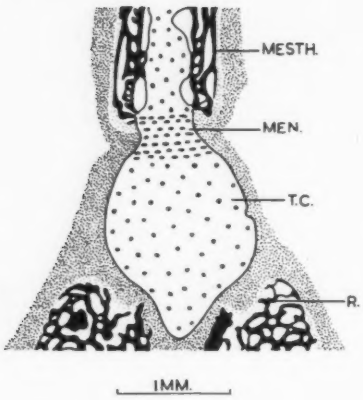

Fig. 16. Transverse section through the trabeculae communis in the 37-day old embryo. *MEN.*, meniscus; *MESTH.*, mesethmoid; *R.*, rostrum; *T.C.*, trabeculae communis.

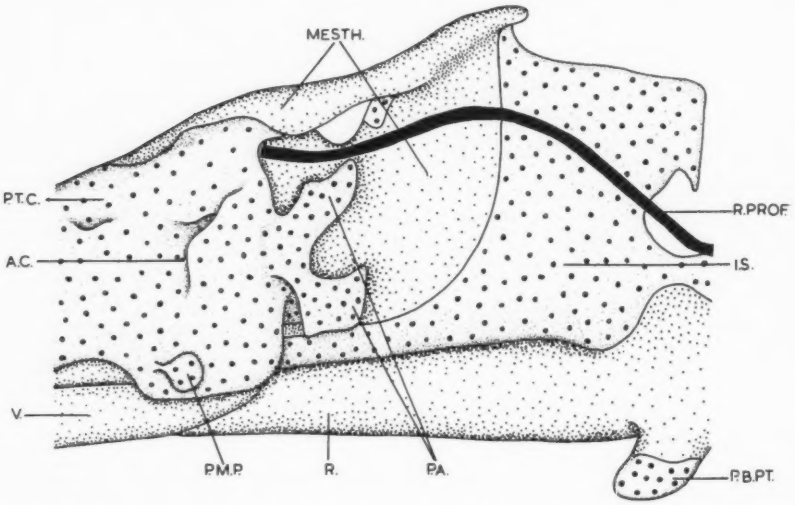

Fig. 17. Graphic reconstruction of the posterior division of the nasal capsule and the interorbital septum in the 37-day old embryo. × 5. Lateral aspect. *A.C.*, aditus conchae; *I.S.*, inter-orbital septum; *MESTH.*, mesethmoid; *P.A.*, planum antorbitale; *P.BPT.*, processus basipterygoideus; *P.M.P.*, processus maxillaris posterior; *P.T.C.*, parietotectal cartilage; *R.*, rostrum; *R.PROF.*, ramus profundus; *V.*, vomer.

of cartilage cells, according to KESTEVEN (1942), has taken on the function of a growth meniscus between the presphenoid below and the septum above. In the 37-day stage, *Struthio* shows very little signs of ossification of the trabecular beam into a presphenoid, but the presence of this meniscus indicates that the presphenoid is a definite element. Dorsally the cribriform plate has in some places undergone complete ossification, with both dorsal and ventral surfaces of the alae ossified, and leaving only a slight core of cartilage. Anteriorly, where the mesethmoid supports the nasal process of the premaxillary it is continous

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with the vertical mesethmoid; that part of the mesethmoid closing the anterior fontanelle becomes separated from the vertical mesethmoid by resorption of the cartilage in this area which forms an oval foramen immediately beneath the mesethmoid in this area (Fig. 17). Posterior to this foramen the flattened dorsal part of the septum supports the frontals, and is unossified, while the sphenoid region of the septum has become partially ossified to form the "orbitosphenoid". Dorso-laterally in the septum there is a groove in which lie the olfactory nerves.

As has been indicated the septum does not ossify from two centres, the boundary, however, between the vertical mesethmoid and "orbitosphenoid" is indicated by the cartilaginous planum antorbitale (lamina orbitonasalis), which, up to the 37-day stage, is still connected to the posterior border of the parietotectal cartilage (Fig. 17) and is loosely connected to the septum by connective tissue. Anterior to this point of attachment is the vertical mesethmoid and posterior to it the "orbitosphenoid".

In the adult the planum antorbitale loses its connexion with the parietotectal cartilage and becomes ossified, forming a preorbital ridge, that may or may not become synostotically connected with the bony septum. The extent of the ossification of the septum anterior to the preorbital ridge is variable; in most of the adults studied it is cartilaginous, forming the nasal septum. Posteriorly the septum may become synostotically connected with the pleurosphenoid and basisphenoid.

On the ventral surface of the processus orbitalis anterior the lacrimal has an oblong groove into which fits the processus maxillaris posterior (Figs. 13, 14 and 15). In the embryonic stages sectioned this element remains cartilaginous and is in synchondrosis with the parietotectal cartilage, and in syndesmosis with the lacrimal. In all the adults studied this element has become ossified (Plate 2 B) and loses its connexion with the parietotectal cartilage. The function of such an element is difficult to imagine, as it is in no way associated with the nasal capsule after it has lost its connexion with it and is in no way related to the kinesis.

(b) Postorbital Cartilage

The postorbital cartilage becomes ossified as the pleurosphenoid. Ossification commences along the anteromedial margin and in the 27-day stage it is limited to this margin. By the 34th-day the ossification occupies the greater portion of the cartilage extending from the frontal to the foramen prooticum in the vertical plane, and extending for the greater part of the horizontal plane, leaving a small anterior and posterior unossified region. The medial and lateral surfaces are unequally ossified, with the lateral surface being more extensively ossified (Figs. 18 and 19). The pleurosphenoid in this stage is suturally con-

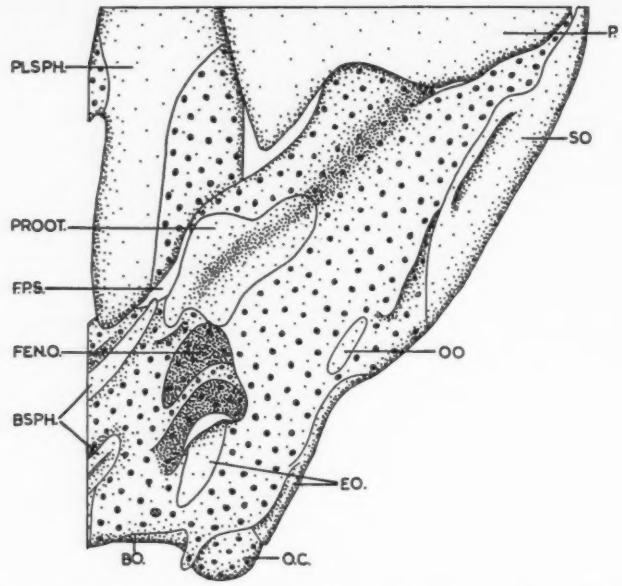

Fig. 18. Graphic reconstruction of the auditory region in the 34-day old embryo. × 5. Lateral aspect. *BO.*, basioccipital; *BSPH.*, basisphenoid; *EO.*, exoccipital; *FEN.O.*, fenestra ovalis; *F.P.S.*, foramen prooticum spurium; *O.C.*, occipital condyle; *OO.*, opisthotic; *P.*, parietal; *PLSPH.*, pleurosphenoid; *PROOT.*, prootic; *SO.*, supraoccipital.

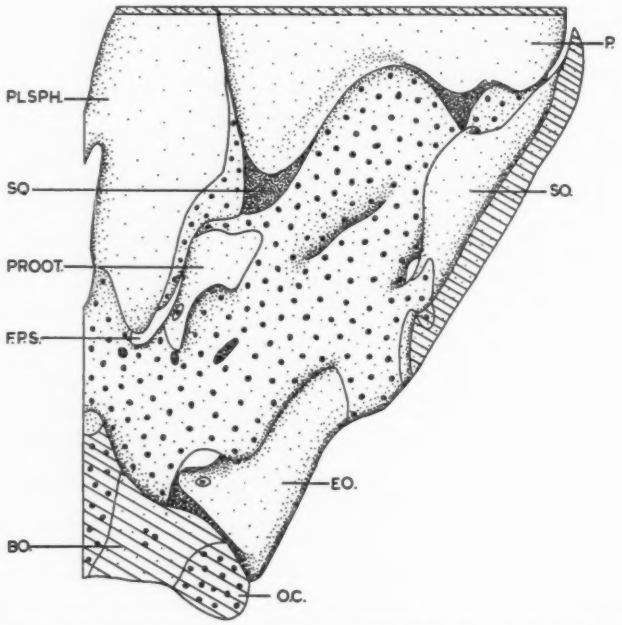

Fig. 19. Graphic reconstruction of the auditory capsule in the 34-day old embryo. × 5. Medial aspect. *BO.*, basioccipital; *EO.*, exoccipital; *F.P.S.*, foramen prooticum spurium; *O.C.*, occipital condyle; *P.*, parietal; *PLSPH.*, pleurosphenoid; *PROOT.*, prootic; *SO.*, supraoccipital; *SQ.*, squamosal.

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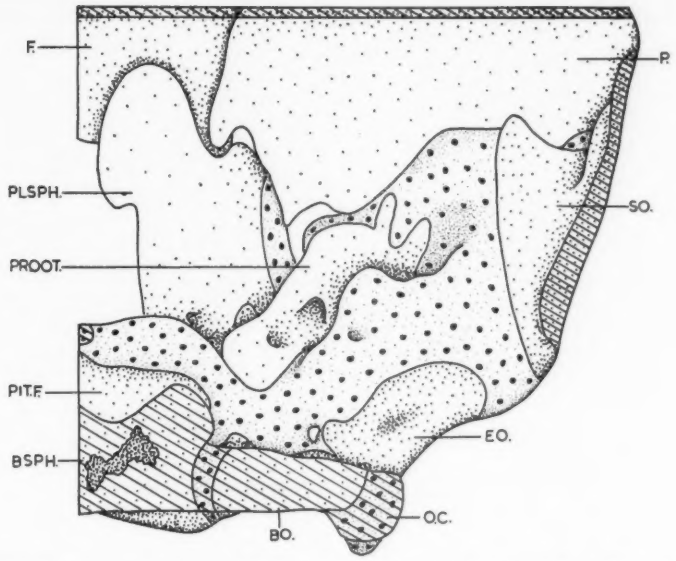

Fig. 20. Graphic reconstruction of the auditory capsule in the 37-day old embryo. × 5. Medial aspect. *BO.*, basioccipital; *BSPH.*, basisphenoid; *EO.*, exoccipital; *F.*, frontal; *O.C.*, occipital condyle; *P.*, parietal; *PIT.F.*, pituitary fossa; *PLSPH.*, pleurosphenoid; *PROOT.*, prootic; *SO.*, supraoccipital.

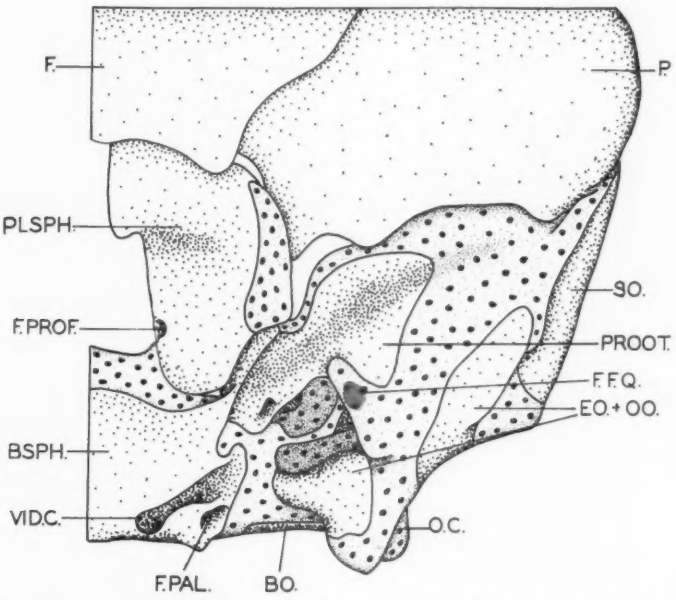

Fig. 21. Graphic reconstruction of the auditory capsule in the 37-day old embryo. × 5. Lateral aspect. *BO.*, basioccipital; *BSPH.*, basisphenoid; *EO* + *OO.*, exoccipital + opisthotic; *F.*, frontal; *F.F.Q.*, facet for quadrate; *F.PAL.*, foramen for r.palatinus; *F.PROF.*, foramen profundum; *O.C.*, occipital condyle; *P.*, parietal; *PROOT.*, prootic; *PLSPH.*, pleurosphenoid; *SO.*, supraoccipital; *VID.C.*, Vidian canal.

nected to the frontal and parietal dorsally. Posterior to the foramen prooticum the postorbital cartilage is unossified and is connected to the prootic region of the auditory capsule by means of dense connective tissue.

In the next stage (37 days) the postorbital cartilage, pila antotica spuria and pila antotica vera are completely ossified, except for the postero-lateral margin of the former, to form the pleurosphenoid (Figs. 20 and 21). Dorsally it is in sutural union with the frontal and parietal. The dorsal margin of the foramen prooticum is completely ossified. The middle cartilaginous plate interposed between the lateral and medial laminae of the developing pleurosphenoid has, for the greater part, been resorbed. There still persists, however, a narrow band of cartilage posteriorly (Figs. 20 and 21) which is the remnant of the postorbital cartilage as yet not showing signs of perichondral ossification.

In the adult the pleurosphenoid is concave medially and convex laterally. The sutures persist between the pleurosphenoid and the frontal and parietal. The suture between the squamosal and pleurosphenoid is lost through synostosis of these two elements. The suture between the pleurosphenoid and basisphenoid is, in most cases, lost through synostosis; in a few, however, it is present and extends in an arc between the prootic foramen and the ossified suprapolar cartilages of the basisphenoid.

In all the embryonic stages the postorbital cartilage or pleurosphenoid is distinguished by the presence of the foramen profundum situated at its antero-medio-ventral corner, and by the notch at the postero-ventral margin, which forms the dorsal surface of the foramen prooticum.

AUDITORY AND OCCIPITAL REGIONS

(a) Occipital Region

The occipital region of the chondrocranium is the first to show signs of ossification. In the 21-day stage four small areas are noticeable (Fig. 4). The basioccipital arises from two centres situated on either side of the midline in front of the margin of the foramen magnum (Fig. 4). In this stage the bone possesses only an outer lamina, the inner lamina developing later. The exoccipitals are small in extent and occupy antero-lateral positions on the margin of the foramen magnum (Fig. 4). They possess an inner and outer lamina pierced by the foramen for nerves IX and X, (foramen vago-glossopharyngeum). The ossification of the supraoccipital from the tectum synoticum has not yet started.

In the 34-day stage the basioccipital has increased considerably in extent and the two separate centres of ossification have united to form a median ventral plate (Fig. 5). There are two laminae separated by the cartilaginous plate. These two laminae encroach upon the cartilaginous condyle from above and below. The exoccipital has increased considerably in extent superficially, with

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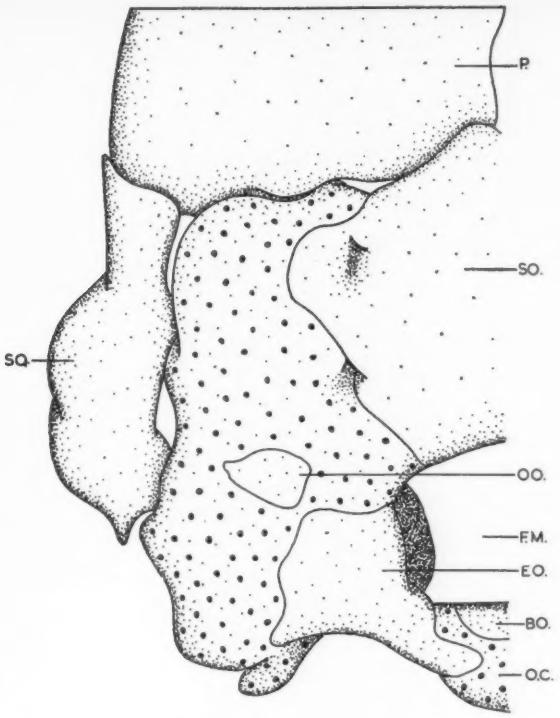

Fig. 22. Contour reconstruction of the skull in the 34-day old embryo. × 5. Posterior aspect. *BO.*, basioccipital; *EO.*, exoccipital; *F.M.*, foramen magnum; *O.C.*, occipital condyle; *OO.*, opisthotic; *P.*, parietal; *SO.*, supraoccipital; *SQ.*, squamosal.

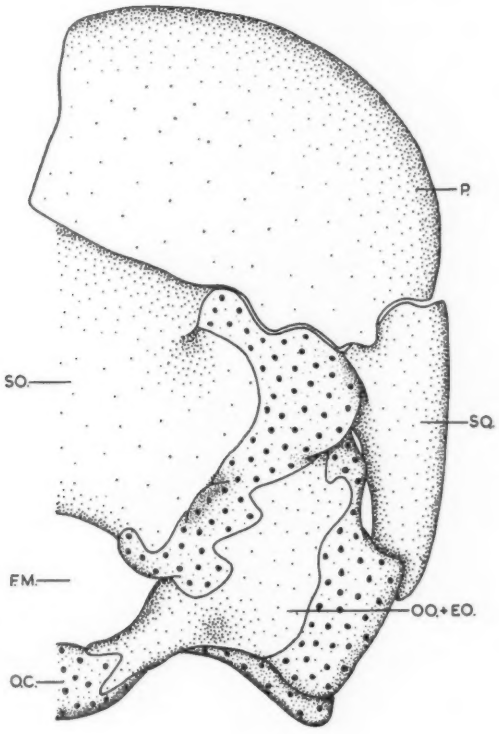

Fig. 23. Contour reconstruction of the skull of the 37-day old embryo. × 5. Posterior aspect. *F.M.*, foramen magnum; *O.C.*, occipital condyle; *OO + EO.*, opisthotic + exoccipital; *P.*, parietal; *SO.*, supraoccipital; *SQ.*, squamosal.

the cartilage between the laminae persisting. There is a posteromedially directed spur of the exoccipital that lies lateral to the condyle which, together with the basioccipital, ossifies the condyle (Fig. 22). The last of the occipital bones is the supraoccipital which begins as a median ossification on the dorsal margin of the foramen magnum and increases rapidly in extent dorsally and forms with the parietal the lambdoidal suture.

By the 37-day stage (Fig. 23) the occipital elements have increased considerably in extent, and the cartilage between the bony laminae has, for the greater part, disappeared. In the adult the sutures are absent.

(b) Auditory Region

The auditory region becomes ossified late in ontogeny, commencing just prior to the 34-day stage. There are two areas of ossification, resulting in two periotic bones, namely, prootic and opisthotic. The former is characteristically situated in the anterior region of the capsule and begins at the margin of the foramen prooticum.

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At the 34th day part of the ventral margin of the foramen prooticum is ossified, the centre extending ventrally to the dorsal rim of the fenestra ovalis (Fig. 18). The lateral and medial laminae are unequal in extent, the former covering about twice the area of cartilage covered by the latter (Figs. 18 and 19). The second centre of ossification is the opisthotic which is posteriorly situated near the dorso-lateral angle of the developing exoccipital (Fig. 18). As with the prootic, the opisthotic has a greater area of ossification laterally than medially, and in both, the laminae are still separated by intervening cartilage. At the 37-day stage the prootic has increased considerably in extent both laterally and medially. It now forms the complete ventral border of the foramen prooticum and the antero-dorsal margin of the fenestra ovalis (Fig. 21). Posteriorly it approaches the exoccipital, being separated from it by a cartilaginous band approximately 8 mm. wide. Posterior to the fenestra ovalis the prootic forms an accessory articulatory surface for the processus oticus of the quadrate; the facet is capped with cartilage, and in osseous continuity with the body of the prootic (Figs. 21 and 24). Medially the prootic is relatively extensive and is pierced by the foramen faciale and foramen acusticum (Fig. 20). The opisthotic has now lost its separate identity having been incorporated into the exoccipital by appositional growth from the two centres (Fig. 21).

PARASPHENOID AND BASISPHENOID

The parasphenoid is dealt with here for the sake of convenience, because of its relation with the basisphenoid.

Up to the 15-day stage there has neither been ossification of the basisphenoid cartilage nor development of the parasphenoid. At the 14-day stage, ventral to

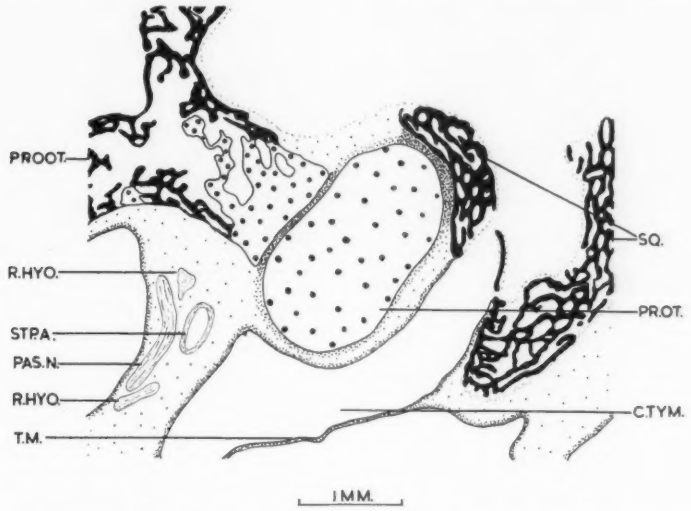

Fig. 24. Transverse section, illustrating the relation between the processus oticus and squamosal and prootic, in the 37-day old embryo. *C.TYM.*, cavum tympanicum; *PAS.N.*, parasympathetic nerve; *PROOT.*, prootic; *PR.OT.*, processus oticus; *R.HYO.*, ramus hyomandibularis; *SQ.*, squamosal; *STP.A.*, stapedial artery; *T.M.*, tympanic membrane.

the fused trabeculae, there appears an accumulation of osteogenetic cells in the mesenchyme condensation, and from these cells the parasphenoid develops. In the 15 day stage the parasphenoid has made its appearance as a long thin rod lying beneath the septum and terminating posteriorly ventral to the basisphenoidal cartilage in the region of the pituitary fossa.

In the next three stages, 17, 19 and 20 days, the posterior end of the parasphenoid has spread out laterally, but has not extended very much further backwards; it becomes closely applied to the overlying cartilage, in the region of the pituitary fossa and carotid foramina. In this region there is a massing of osteoblasts which lie against the cartilage, the fibrous perichondrium having disappeared. From the 19-day stage ossification of the cartilaginous cranial base proceeds rapidly, first appearing as a slight ossification of the infrapolar cartilages. In the 21-day stage ossification of the cartilage has spread laterally, and posteriorly. In this stage the processus basipterygoideus has become ossified at its base, and the anterior wall of the lateral carotid foramen shows signs of superficial ossification. By the 27th day, ossification has progressed considerably, with the basipterygoid processes now enclosed in an osseous sheath with a persistent cartilaginous core; distally the process is capped with cartilage continuous with the core.

The floor of the pituitary fossa in the 27-day stage shows very definite signs of ossification (Fig. 25) which was first apparent in the 21-day stage, though very slightly. In the 27-day old embryo the basal plate in the region of

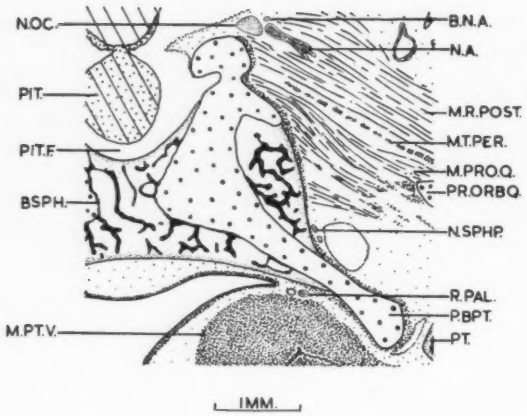

Fig. 25. Transverse section through the cranial base in the region of the pituitary fossa in the 27-day old embryo. *B.N.A.*, branch of n.abducens to m.pyramidalis and m.quadratus; *BSPH.*, basisphenoid; *M.PRO.Q.*, m.protractor quadrati; *M.PT.V.*, m.pterygoideus ventralis; *M.R.POST.*, m.rectus posterior; *M.T.PER.*, m.tensor periorbitae; *N.A.*, nervus abducens; *N.OC.*, n.oculomotorius; *N.SPHP.*, n.sphenopalatinus; *P.BPT.*, processus basipterygoideus; *PIT.*, pituitary; *PIT.F.*, pituitary fossa; *PR.ORBQ.*, processus orbitoquadratus; *PT.*, pterygoid; *R.PAL.*, ramus palatinus.

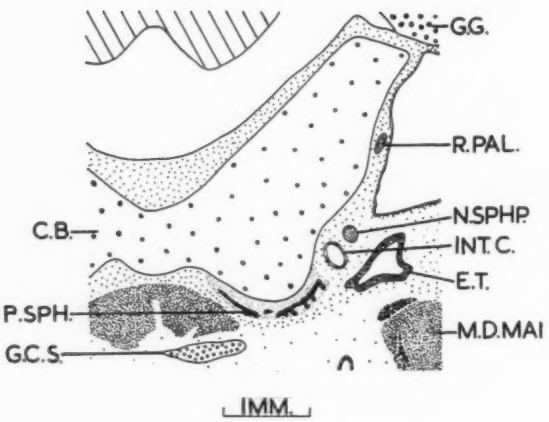

Fig. 26. Transverse section of the cranial base illustrating the posterior process of the basisphenoid in the 27-day old embryo. *C.B.*, cranial base; *E.T.*, eustachian tube; *G.C.S.*, ganglion cervicale supremum; *G.G.*, ganglion gasseri; *INT.C.*, internal carotid; *M.D.MAN.*, m.depressor mandibulae; *N.SPHP.*, n.sphenopalatinus; *P.SPH.*, parasphenoid; *R.PAL.*, ramus palatinus.

the pituitary fossa is covered by a dorsal and a ventral lamina enclosing a cartilaginous plate; these two lamina are in contiguity by way of the carotid foramina.

Posterior to the carotid foramen and the processus basipterygoideus, the perichondral ossification of the cranial base becomes divided into two distinct posteriorly directed processes separated by a medial notch. At the broadest part of the parasphenoid, in the region of the processus basipterygoideus, the cranial base, as well as the processus basipterygoideus ventrally, become ossified though being closely applied to the ventral surface of the cranial base, are separated from it by means of dense connective tissue (Fig. 26). The r.palatinus passes ventral to the processus basipterygoideus, and the internal carotid enters the cranial floor by way of the internal carotid foramen.

In this stage it is quite obvious that the cranial base is made up of three

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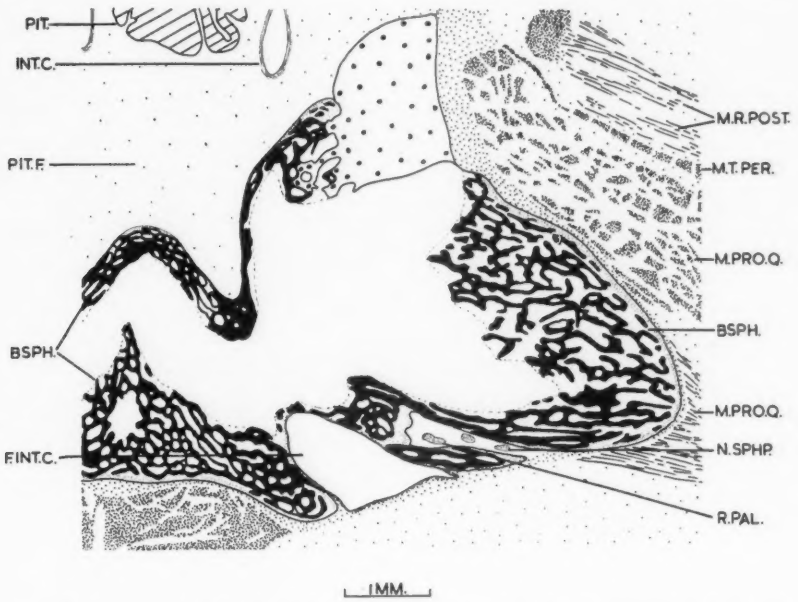

Fig. 27. Transverse section of the basisphenoid illustrating the foramen for the n.sphenopalatinus, and r.palatinus in the 37-day old embryo. *BSPH.*, basisphenoid; *F.INT.C.*, foramen for internal carotid; *INT.C.*, internal carotid *M.PROQ.*, m.protractor quadrati; *M.R.POST.*, m.rectus posterior; *M.T.PER.*, m.tensor periorbitae; *N.SPHP.*, n.sphenopalatinus; *PIT.*, pituitary; *PIT.F.*, pituitary fossa; *R.PAL.*, r.palatinus.

elements. The basioccipital region of the primordial cranial base is developed only of substitution bone, while the anterior region of the primordial cranial base, on which the parasphenoid develops is ossified perichondrally, but is mixed in its origin, developing as substitution bone dorsally and ossifying from membrane bone ventrally.

In the 34 and 37-day stages, the basic difference from the 27-day stage is one of extent of the laminae involved in the ossification of the basal plate. In the 34-day old embryo there is still a layer of cartilage between the laminae, while in the 37-day stage the cartilaginous core has also been replaced by bone. The suprapolar cartilages still remain unossified, while the infrapolar cartilages, pituitary fossa and the walls of the carotid foramina are completely ossified. The processus basipterygoideus is completely osseus except for the distal extremity which remains cartilaginous, being involved in a synovial arthrosis with the pterygoid.

The ventral margin of the foramen prooticum is formed partially by a postero-dorsally directed process of the basisphenoid, which terminates immediately ventral to the ventrolateral margin of the pleurosphenoid (Fig. 18). In the body of the basisphenoid + parasphenoid there is a large air-space which,

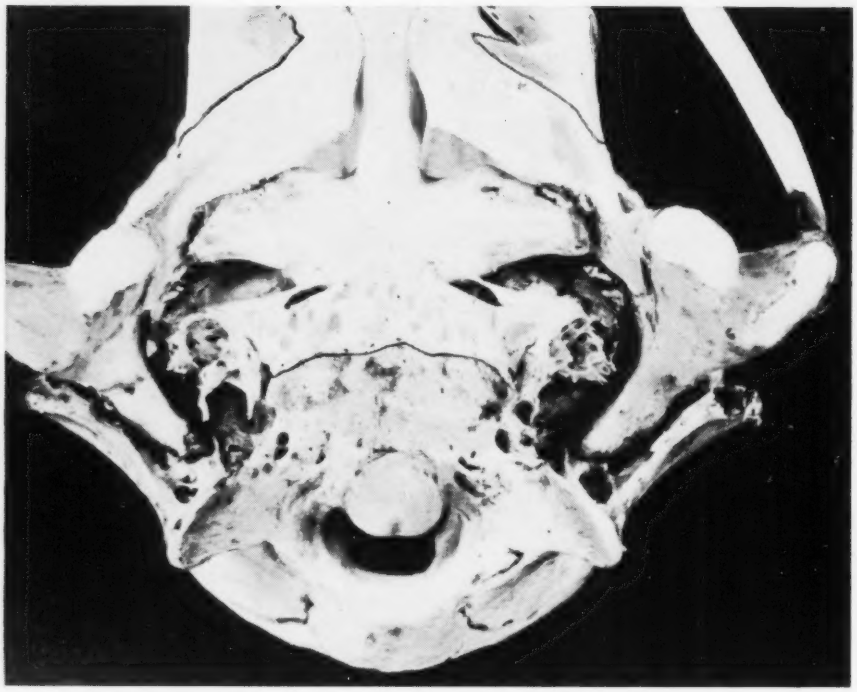

Plate 3. Photograph of the cranial base of the adult illustrating the dividing line between the basisphenoid and basioccipital.

together with the spongy nature of the bone itself, makes it highly pneumatic (Fig. 27). The parasphenoidal rostrum is also very highly pneumatized.

The antero-dorsal margin of the basisphenoid is separated from the overlying developing pleurosphenoid by a zone of cartilage showing the characteristic rearrangement of cells, so that their axes lie horizontal, thus forming a cartilaginous strip.

With the enlargement of both the basisphenoid + parasphenoid and the basioccipital the intervening cartilage has, by the 37th day, been reduced to a thin strip, approximately 2.5 mm. wide (Fig. 20), which will eventually be lost in the adult, thus forming a completely ossified cranial base. However the boundary between the two elements is distinguishable in the adult as a well pronounced groove in some cases and a ridge in others (Plate 3).

In both the 34th and 37th day old embryos the carotis interna leaves the cranial cavity by way of its own foramen together with a branch of the parasympathetic nerve associated with the r.palatinus (VII). The r.palatinus lies ventral to the processus basipterygoideus and in its course posteriorly it meets with the parasympathetic nerve (n.sphenopalatinus) and both enter a foramen

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(Fig. 27) in the side-wall of the sphenoid (basisphenoid + parasphenoid). This foramen may correspond to the parabasal canal of *Reptilia*.

DISCUSSION OF THE OSSA SUBSTITIENTIA

Previous works describing the ontogeny of the replacement bones of the *Dromaeognathae* are confined to a few authors. W. K. PARKER (1866, 1866 (a) and 1876), and T. J. PARKER (1891) contributed considerably to the knowledge of these bones. Since that time little has been done on the osteo-craniogenesis of the *Dromaeognathae*, except where such work is directly related to the specific problems investigated. Notable among these is PYCRAFT (1900). More or less the same applies to the carinates, with W. K. PARKER having produced the greater number of works. Other authors have added to the knowledge of osteo-craniogenesis of carinates, such as TONKOFF (1900) on the fowl, SUSCHKIN (1899) on *Tinnunculus* and KESTEVEN (1942) on *Anas* in general with special reference to the emu.

THE SEPTUM

The septum and the flattened dorsal plate—the cribriform plate—become ossified as the ethmoid in the region of the lacrimal (PARKER, 1866). PYCRAFT (1900-1901) is of the opinion that in the tinamou the dorsal border of the mesethmoid which supports the nasals and frontals is a separate element and he calls it the ectoethmoid, and refers to the alae of the septum (PARKER, 1866) as the inferior aliethmoids when ossified. In *Apteryx* there is a single septal element, the mesethmoid which is the only ossification of the ethmoid region where it spreads anteriorly into the nasal septum (DE BEER, 1937). The orbitosphenoid in *Apteryx* is absent (PARKER, 1891).

KESTEVEN (1942) describes the septum of the emu as undergoing osteoblastic activity which is ectochondral (perichondral) in nature, and which takes place on either side of the septum, while the cartilage enclosed between the two laminae becomes slowly reduced, until it is finally replaced by bone. The centre of ossification of the septum spreads anteriorly to the hinder margin of the nasal septum, and posteriorly so as to ossify the whole of the septum. Dorsally the ossification does not reach the roofing bones, and ventrally the septum is separated from the already ossified trabeculae by a cartilaginous strip, thus separating the septum from the presphenoid.

All birds possess a septum which, with the exception of the *Ratitae* + *Crypturi* develops a secondary split, the fissura cranio-facialis, which becomes associated with kinesis (MARINELLI, 1936). The presence of this fissura is not the criterion for kinesis in the class *Aves*, but can be regarded as having been developed to assist in kinesis.

As has been seen there is in *Struthio* a single ossification centre of the septum

which gives rise to the mesethmoid (presphenoid, DE BEER, 1937) anterior to the planum antorbitale and to the secondarily single orbitosphenoid posteriorly. DE VILLIERS (1946) leads one to believe that these two regions are ossified from two separate centres by referring to "... the synostotic continuity of the mesethmoid and orbitosphenoid." This is quite definitely not the case in *Struthio*. In *Apteryx* the orbitosphenoid is absent (PARKER, 1891); and BÜTSCHLI (1910) mentions that in birds there are a pair of small ossifications in the orbital septum, which are homologous, with the orbitosphenoids of fishes, but which soon lose their separate identity and become fused with the neighbouring bones. BÜTSCHLI (1910) further maintains that in birds the boundary between the orbital and nasal septa is indicated by a replacing bone which he refers to as the mesethmoid. This mesethmoid grows backwards in the cartilaginous orbital septum. However, in most birds, the orbital septum becomes ossified from two centres on either side and these represent the orbitosphenoids. It is possible that in *Struthio* the true orbitosphenoids are lost, in which case the posterior extension of the mesethmoid into the interorbital septum can only be referred to as the orbitosphenoid, topographically, and is in no way homologous with the true orbitosphenoids of other birds. On the other hand the orbitosphenoid centres of ossification may have been incorporated into the mesethmoid. GOODRICH (1930) considers the orbitosphenoids of the *Labyrinthodontia* as having been incorporated into the pleurosphenoid.

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Curiously enough, the condition of the ethmoidal region in *Struthio* and other *Dromaeognathae* resembles that obtaining in the *Anura* in that the mesethmoid appears on the dorsal surface. Further, there is similarity in the ossification of the sphenethmoidal region in *Struthio* and *Anura*. In the *Anura* when the septum nasi ossifies to form the mesethmoid and the anterior part of the os en ceinture, the posterior part of the os en ceinture forms the "orbitosphenoid". Similarly, in *Struthio* the ossification of the septum, though single, forms the mesethmoid anterior to the planum antorbitale, and the "orbitosphenoid" posterior to it.

Besides the mesethmoid there is in *Struthio*, a separate lateral element, the lateral ethmoid, derived by ossification of the planum antorbitale. It was not possible to determine whether the planum antorbitale ossified separately, or was ossified from the mesethmoid, as it was still cartilaginous in the 37-day old embryo. DE BEER (1937) points out that as the planum antorbitale ossifies in fishes, but not in most living amphibians and reptiles, the condition in birds, where the ossification forms the anterior wall of the orbit, should be referred to as the ectethmoid, because in birds it has possibly evolved independently and has not been inherited from the *Osteichthyes*. This ossification of the planum is well developed in *Struthio* as well as in *Rhea* and *Dromiceius* (PARKER, 1866) and also in *Apteryx* (PARKER, 1891).

In the region of the lacrimal, and syndesmotically connected with it, there is

a small replacing element derived by ossification of the processus maxillaris posterior. It possibly represents the lacrimo-palatine ossicle or "os uncinatum" (PYCRAFT, 1900). DE BEER (1937) mentions that there is a lacrimo-palatine ossicle or uncinate bone in many birds, extending from the ectethmoid to the jugal or to the palatine. He tentatively suggests that it is segmented off from the ectethmoid. BRAND (1839) described the presence of a small bone ventral to the lacrimal in *Tubinares, Steganopodes* and *Musophagi* and termed it the "os uncinatum". It has also been described for *Sterna* (Selenka). This small bone in *Struthio* can therefore be regarded as the homologue of the "os uncinatum" (lacrimo-palatine ossicle).

THE PLEUROSPHENOID

In *Struthio* the pleurosphenoid ossifies from the postorbital cartilage, pila antotica spuria, and the considerably reduced pila antotica vera, and lies between the r.profundus and truncus maxillo-mandibularis. PARKER (1866) was fully justified in calling this bone the alisphenoid because of its relation to these nerves. However, the alisphenoid (= epipterygoid), though having the same relationship with Va and Vb and c, is ossified from the processus ascendens of the palato-quadrate bar.

KESTEVEN (1942) states that the postorbital cartilage in the emu becomes ossified from several centres on the cerebral surface of the lamina alisphenoidea, thus forming the alisphenoid. In *Gallus,* the pleurosphenoid ossifies in the postorbital cartilage and pila antotica spuria (DE BEER, 1937). DE BEER (1937), however, states on p. 439, "In birds the formation of the pila antotica spuria leads to the ossification of the pleurosphenoid between V_1 and V_2 . . ." This is in fact not the condition in *Struthio* nor in *Gallus* as stated by DE BEER.

THE OCCIPITAL AND AUDITORY REGIONS AND THE CRANIAL BASE

The occipital region of *Struthio* differs little from that of other birds; all three elements are present, and the occipital condyle is formed from both the exoccipitals and the basioccipital. In *Gallus* (DE BEER, 1937) the condyle is ossified from the basioccipital. The supraoccipital ossifies from a single centre and spreads laterally as in *Apteryx* (T. J. PARKER, 1891), while in *Gallus,* according to PARKER (1869) it arises as paired anlagen in the tectum synoticum. PARKER (1866) was unable to ascertain whether the supraoccipital ossified as an azygous or paired element in the "*Struthionidae*" and mentions that *Turdus* is the only bird he found to possess an original azygous supraoccipital. An unpaired supraoccipital also occurs in *Tinnunculus* (SUSCHKIN, 1899), while in *Larus ridibundus* (PARKER, 1866) the supraoccipital is laid down from four centres, which VERSLUIJS (1924) regards as being an adaptation to the increase

in the size of the brain. GADOW (1891) considers that the lateral parts of the supraoccipital represent the epiotica.

Cranial Base

As has already been described, the primordial cranial base ossifies perichondrally into an anterior basisphenoid and a posterior basioccipital. Underlying the basisphenoid and the fused trabeculae is the parasphenoid, which, though a membrane bone, invades the cartilage from below, thus giving rise to a mixed bone called by DE BEER (1937), the sphenoid. This fusion of the basisphenoid and the parasphenoid is not confined to *Struthio,* but is also reported for *Gallus* (DE BEER, 1926) and is found in all reptiles.

Up to and including the 27-day old embryo, the parasphenoid and basisphenoid are fairly easily distinguishable from each other, but in the 34 and 37-day old embryos, it is impossible to distinguish the parasphenoid from the basisphenoid except for the rostrum. However, in the *Reptilia* even though the parasphenoid and basisphenoid are fused, the presence of a parabasal or Vidian canal between these two elements indicates the dividing line. This canal allows for the passage of the internal carotid and r.palatinus (VII) (VAN PLETZEN, 1946). TOERIEN (1950) mentions quite definitely that at the base of the processus basipterygoideus the line of fusion is indicated by the parabasal canals. He further maintains that the parasphenoid forms part of the ventral aspect of the processus basipterygoidi. This is also true for *Struthio.*

DE VILLIERS (1939, p. 335) sums up the position in *Lacertilia,* which could also, to a certain degree, be applied to *Struthio,* as follows:

"Bekanntlich kommt es bei Lazertilian zur Bildung eines Mischknochens, indem der Deckknochen, das Parasphenoid, die dorsal vor ihm gelegene knorpelige Schädelbasis, also Mutterboden des Basisphenoid und Basioccipitale, invahiert. Dies alles läßt sich am erwachsenen Schädel leicht feststellen, obgleich, wegen gegenseitiger Verschmelzung, die Grenzen zwischen Basioccipitale, Basisphenoid und Parasphenoid ebensowenig micro- wie makroskopisch deutlich nachzuweisen sind."

The boundary, in *Struthio,* between the basisphenoid and parasphenoid is not clear, especially in the late stages of development.

The course of the internal carotid and r.palatinus VII does not indicate the boundary between the parasphenoid and the basisphenoid as it does in the reptiles. The internal carotid does not pass through the same foramen as that for the r.palatinus and the n.sphenopalatinus, but through its own foramen. The foramen through which the r.palatinus and n.spenopalatinus pass in *Struthio* is in all probability not homologous with the parabasal canal of reptiles.

The "basitemporal" element as described by PARKER (1866) for *Struthio, Rhea* and *Dromiceius* and by PARKER (1891) for *Apteryx* corresponds to the

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two posteriorly directed processes, connected to the cranial base by dense connective tissue as has been described for the 27 day old embryo. The "basitemporal" elements according to BÜTSCHLI (1910), WIEDERSHEIM (1909) and STRESEMANN (1927—1934) are laid down as paired centres of ossification; and a median unpaired element forms the parasphenoidal rostrum lying ventral to the fused trabeculae. This condition seems to be general for birds as the presence of "basitemporal" elements are reported for *Gallus* (PARKER, 1869) and TONKOFF (1900); *Tinnunculus* (SUSCHKIN, 1899) and *Anser* (STRESEMANN, 1927—1934). However, the "basitemporals" are topographic names for the posterior processes of the parasphenoid, which do not arise from separate centres of ossification, and are in synostotic continuity with the parasphenoidal rostrum, and are in reality nothing but parasphenoid.

KESTEVEN (1942) made a detailed study of the ossification of the chondrocranium in the emu and fifteen other species of birds. Of the cranial base he has the following to say (1942, p. 235): "The basitemporal bones and the parasphenoid rostrum are extraperichondral portions of the basi- and presphenoid bones respectively, and not membrane bones as has hithertofore been believed." How far this is true for the emu and the other birds studied is difficult to say; it does not, however, fit the case for *Struthio* as the rostrum and the part ventral to the basipterygoid are ossified in a manner characteristic of membrane bones, even though in the region of the basipterygoid processes, the membrane bone is not separated from the overlying cartilage by connective tissue. However, more posteriorly the connective tissue is present. In *Acontias* the connective tissue dividing line between the basisphenoid and the parasphenoid is by no means continuous, but both bones are frequently joined by bony bridges (DE VILLIERS, 1939). In *Sphenodon* the parasphenoid is completely separated from the basisphenoid by connective tissue (FUCHS, 1910).

The primordial cranial base of *Rhynchocephalia* is similar to that of *Struthio, Dromiceius, Spheniscus* and *Nyctisyrigmus,* in not possessing a basicranial fenestra. FUCHS (1910) considered that because *Sphenodon* has a complete cranial base the posterior portion of the parasphenoid has no functional significance and is therefore rudimentary.

The condition in *Struthio* could possibly be regarded as being primitive especially in view of the fact that the basicranial fenestra is also lacking in the *Crocodilia, Anura* and *Dipnoi* (FRANK, 1954). This contention is not justified, seeing that these three groups are all end groups. Before one calls *Struthio* primitive, is is also wise to consider the possibility of its being neotenic, as it is, as DE VILLIERS (1946) points out, exceedingly difficult to distinguish between a neotenic and a truly primitive condition.

In *Struthio* and other *Dromaeognathae* the primordial cranial base is complete; it is, however, complete only for a short time in *Pyromelana* (ENGELBRECHT, in press) and in *Tinnunculus* (SUSCHKIN, 1899) because a basicranial

fenestra develops in it by resorption of the cartilage. This may lead one to believe that the condition of the cranial base in *Struthio* is neotenic and not primitive.

The condition of the parasphenoid in *Struthio* could possibly be explained in a similar way as was done for *Sphenodon,* being reduced because its function as a floor to the basicranial fenestra is lost.

The Auditory Capsule

The auditory capsule is ossified as the prootic and opisthotic. PARKER (1866) mentions the presence of these two bones in *Struthio, Rhea* and *Dromiceius,* while in *Casuarius* he describes in addition the presence of a third bone, the pterotic, which is regarded by DE BEER (1937) as being the epiotic as it appears on the dorsal part of the posterior semicircular canal and becomes fused with the exoccipital. PARKER (1891) describes only two periotic bones for *Apteryx:* the epiotic being absent (MARINELLI, 1936 and STRESEMANN, 1927—1934). GADOW (1891) considers that the lateral part of the supraoccipital corresponds with the epiotic.

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THE QUADRATE (OS QUADRATUM)

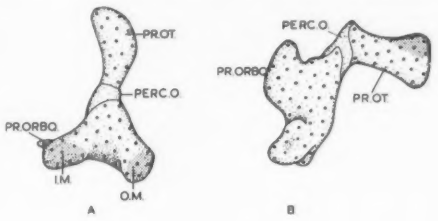
(a) Ontogeny

FRANK (1954) gives a brief account of the formation of the pars quadrata up to and including the 15.3 mm. embryo, the quadrate in this latter stage resembling that of the adult condition with the processus oticus which abuts against the prootic region of the auditory capsule. Projecting medially from the main body of the element is a broad flat processus orbitoquadratus connected to the otic region by the m.protractor quadrati.

Early in ontogeny the pars quadrata develops two articulory facets for the pars articularis of Meckel's cartilage. With the formation of the quadratojugal in the 15-day stage, the laterally directed process of the pars quadrata develops distally an excavation into which fits the posterior end of the quadratojugal. This laterally directed process has on its ventral surface, the lateral transverse articulatory surface with Meckel's cartilage (Fig. 28).

Ossification of the pars quadrata commences between the 20th and 21st day of development and is perichondral in nature, beginning, as a localized area, at the base of the processus oticus (Fig. 28).

In the 27-day stage the process of ossification has advanced considerably, not only in the formation of an osseous covering of the shaft of the processus oticus, but also in the reduction of the cartilaginous core (Fig. 29). During the 21st to the 27th day the quadrate has lengthened dorso-ventrally, and has



Figs. 28 A and B. Graphic reconstruction of the pars quadrata of the 21-day old embryo. × 5. a) Ventral aspect. b) Lateral aspect. *I.M.* and *O.M.*, inner and outer articulatory surfaces of the pars quadratum with Meckel's cartilage; *PERC.O.*, perichondral ossification; *PR.ORBQ.*, processus orbitoquadratus; *PR.OT.*, processus oticus.

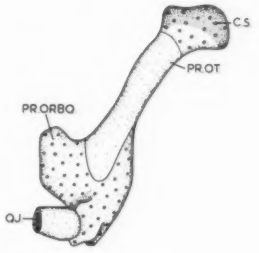
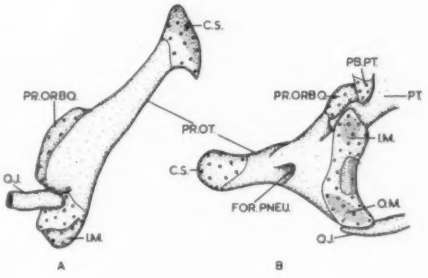


Fig. 29. Graphic reconstruction of the pars quadrata of the 27-day old embryo. × 5. Lateral aspect. *C.S.*, condyle for the articulation with the squamosal; *PR.ORBQ.*, processus orbitoquadratus; *PR.OT.*, processus oticus; *QJ.*, quadratojugal.

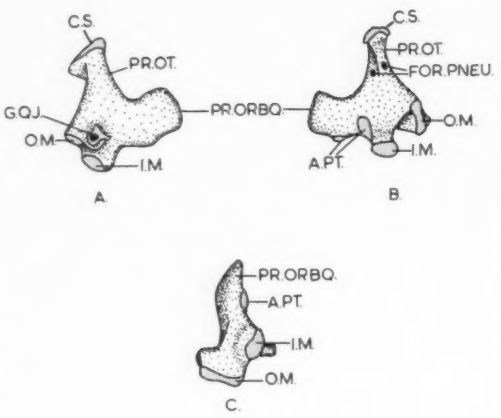


Figs. 30 A and B. Graphic reconstructions of the quadrate in the 34-day old embryo. × 5. a) Lateral aspect of left quadrate. b) Ventral aspect of right quadrate. *C.S.*, single condyle of the quadrate; *FOR.PNEU.*, pneumatic foramen; *I.M.* and *O.M.*, inner and outer articulatory facets of the quadrate with the mandible; *P.BPT.*, processus basipterygoideus; *PR.ORBQ.*, processus orbitoquadratus; *PR.OT.*, processus oticus; *PT.*, pterygoid; *QJ.*, quadratojugal.

straightened (cf. Figs. 28 and 29). By the 34th day the quadrate has, for the greater part, become completely ossified perichondrally (Fig. 30). The central core of cartilage has almost disappeared, being completely absent in the basal part of the processus oticus. In the 37-day stage no further change has taken place except for the degree of ossification; the persistent cartilaginous regions are now confined to the extremities of the bone, being most prominent on the surfaces which articulate with the neurocranium.

(b) The Adult Quadrate

The bone is fully ossified and pneumatized, and has a triradiate structure. The most pronounced of the three processes is the processus orbitoquadratus (processus orbitals of Moller, 1930), which projects anteromedially from the body of the bone, and serves for the attachment of the m.protractor quadrati (m.orbitoquadratus) on its posterior surface.



Figs. 31 A, B and C: Sketches of the adult quadrate (½ natural size). a) Lateral aspect. b) Medial aspect. c) Ventral aspect. *A.PT.*, area of attachment with the pterygoid; *C.S.*, transverse condyle of quadrate for the squamosal; *FOR.PNEU.*, foramen pneumaticum; *G.QJ.*, glenoid facet for the quadratojugal; *I.M.* and *O.M.*, inner and outer articulatory facets for the mandible; *PR.ORBQ.*, processus orbitoquadratus; *PR.OT.*, processus oticus.

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Ventrally there are two articulatory surfaces for the articulation with the articular (Figs. 31 B and C). The outer facet is more or less transversely directed and is not at the same level as the inner facet which is medially placed and divided into two distinct regions: a ventral horizontal region and a lateral vertical region. This arrangement of the facets prevents the lower jaw from moving from side to side.

The processus oticus is relatively short and terminates in a single elongated condyle (Fig. 31 A and B) which is characteristic of the dromaeognathous birds. On the posterior margin of the vertical shaft of the processus oticus are two pneumatic foramina lying one above the other (Fig. 31 B).

Discussion

Considerable importance is attached to the nature of the condyle of the processus oticus of the quadrate. HUXLEY (1867) uses the fact that the condyle is undivided into two distinct heads, as a characteristic of the *Dromaeognathae*. PARKER (1866) in his work on *Struthio, Rhea* and others mentions that in *Struthio* the head of the "quadrate cartilage" shows no "subdivision into an anterior and posterior incudal crus" (1866, p. 120). This singularity of the processus oticus condyle is also applicable to *Rhea, Dromiceius* and *Casuarius* (PARKER, 1866); *Tinamous* (PARKER, 1866a) and particularly *Crypturellus* (DE VILLIERS, 1946).

PARKER (1866a) discusses in greater detail the condition of the processus oticus in *T.robustus*. He states the presence of a single dorsal facet, as the most important character of the quadrate. Further, he mentions that "... its long axis is not parallel with the axis of the head, but oblique ..." (pp. 220—221). The axis of the head possibly refers to the "long axis" or "antero-posterior axis". This condition is similar to that in *Struthio*, which will be further dealt

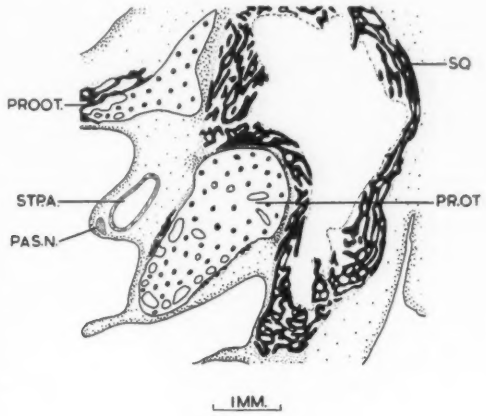

Fig. 32. Transverse section through the processus oticus and squamosal in the 37-day old embryo. *PAS.N.*, parasympathetic nerve; *PROOT.*, prootic; *PR.OT.*, processus oticus; *STP.A.*, stapedial artery; *SQ.*, squamosal.

with in discussing kinesis. An important difference with that of *Struthio* is that for *T.robustus,* (PARKER, 1866a) mentions that "... its corresponding facet on the skull is extended along the side of the prootic..." (p. 221, 1866a), while in *Struthio* the condyle contacts the prootic only posteriorly.

PARKER (1866a), while discussing the processus oticus of *T.robustus,* mentions on p. 221 "... there is no division into an external or anterior head and an internal or posterior. Once above the 'Ostriches' and tinamous, we have the articular condyle divided into two facets". This makes it quite clear that up to this stage *Apteryx* had not yet been studied. HUXLEY (1867), a year later, includes *Apteryx* in his definition of the *Dromaeognathae* as having an undivided head of the quadrate but he could not have examined it personally because PARKER (1891), in his work on *Apteryx,* states quite emphatically, that "*Apteryx*... can by no means be described as having a single headed otic process" (p. 52, 1891). This condition in *Apteryx* is also found in the carinate birds on which there is a medial articulatory head in contact with the prootic and a lateral head in contact with the squamosal as in *Crus, Laro-limicolae, Striges* and *Accipitres* (STRESEMANN, 1927—1934) and *Serricotes, Cinnyris* and *Anthornis* (MOLLER, 1930). These articulatory heads of the processus oticus are in line and at right angles with the longitudinal axis of the neurocranium. It is impossible to state whether there has been a rotation of the processus oticus so that in the *Carinatae* it now lies at right angles to the longitudinal axis of the head, resulting in the formation of a double-headed otic process with the medial facet articulating with the prootic and the lateral facet with the squamosal.

RELATIONS OF THE QUADRATE TO THE SURROUNDING ELEMENTS

The single elongated condyle of the processus oticus is connected by dense connective tissue to the squamosal with which it articulates (Figs. 24 and 32).

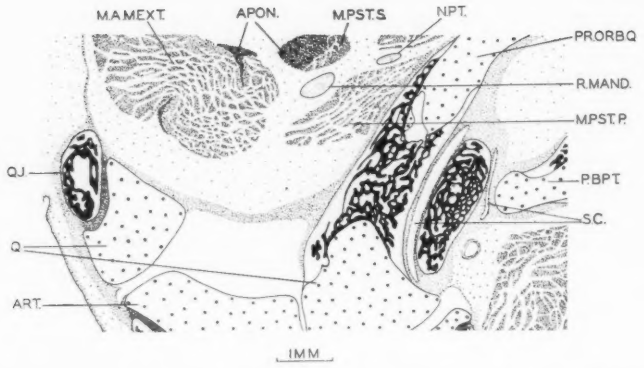

Fig. 33. Transverse section through the quadrate showing its relation to the pterygoid and quadratojugal in the 34-day old embryo. *APON.*, aponeurosis; *ART.*, articular cartilage; *M.A.M.EXT.*, m.adductor mandibulae externus; *M.PST.P.*, m.pseudotemporalis profundus; *M.PSTS.*, m.pseudotemporalis superficialis; *N.PT.*, n.pterygoideus; *P.BPT.*, processus basipterygoideus; *PR.ORBQ.*, processus orbitoquadratus; *Q.*, quadrate; *QJ.*, quadratojugal; *R.MAND.*, r.mandibularis; *S.C.*, synovial cavity.

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There is, however, a second articulation with the prootic (Fig. 24), which is only in contact with the condyle of the processus oticus over a very small area, and on the medial posterior surface of the condyle. The squamosal is grooved on its medial surface to receive the head of the processus oticus, which, in the 37-day stage, is capped with cartilage. The surface of the prootic in contact with the processus oticus is also capped with cartilage (Fig. 24). Though the quadrate of *Struthio* contacts both squamosal and prootic, the processus oticus is not divided into two heads characteristic of "carinate" birds (Brock, 1937).

The nature of the connexion of the processus oticus with the squamosal is such that there is limited movement, which can only be executed inwards towards the middle line. The vertical shaft of the processus oticus is connected to the medial surface of the processus zygomaticus of the squamosal, thus adding stability to the already reduced movement of the quadrate.

The quadratojugal is rigidly connected to the lateral process of the quadrate by dense connective tissue. No movement at all is possible between these two bones. The lateral process of the quadrate is distally excavated to receive the posterior end of the quadratojugal (Figs. 29, 30 and 31) which, when in position, lies flush with the lateral surface of the quadrate. The applied surfaces lie very close to one another and are only separated by a thin layer of connective tissue (Fig. 33).

Separating the quadrate and pterygoid is a synovial cavity (Fig. 33), thus permitting these two bones to have unimpeded movement between them. The medial surface of the quadrate is slightly concave so as to receive the posterior end of the pterygoid which is vertical and slightly convex on its lateral surface (Fig. 33). The synovial cavity develops relatively late in ontogeny, and in the

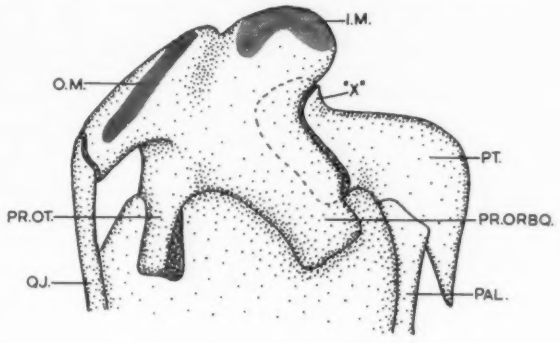

Fig. 34. Sketch of the quadrate and its attachments with the pterygoid and quadratojugal in the adult (seen obliquely). *I.M.*, and *O.M.*, inner and outer articulatory facets for the mandible; *PAL.*, palatine; *PR.ORBQ.*, processus orbitoquadratus; *PR.OT.*, processus oticus; *PT.*, pterygoid; *QJ.*, quadratojugal; *"X"*., strut of pterygoid.

27-day stage that area of the arthrosis which will give rise to the synovial cavity, contains loose connective tissue, on the articular disc, which is continuous with the periosteum of the pterygoid and the perichondrium of the proximal region of the processus orbitoquadratus. By the 34th and 37th day, the synovial cavity is fully formed with neither of the surfaces capped with cartilage, the quadrate having become completely ossified in the region of the arthrosis. The pterygoid, in the late stages of development, and especially in the 37-day stage, shows no signs of developing a cartilaginous cap in the region of the synovial arthrosis.

The ventral part of the pterygoid (indicated by "X" in Fig. 34) forms a buttress against the quadrate which, as will be seen when the problem of kinesis is discussed, is of considerable significance.

KINESIS

De Villiers (1946) has given a full account of the kinesis in *Struthio*, as it appears from the study of a prepared adult skull. Naturally there are certain reservations and discrepancies caused by the lack of embryonic material. The present investigation provides the necessary additional knowledge, especially that concerning the arthroses, and the histology of the joints directly involved during kinesis.

The kinesis of the skull of the dromaeognathic types is restricted, *Apteryx* being apparently akinetic (de Villiers, 1946). It appears that the lifting of the beak in the typical mesokinetic manner does not exist in the *Dromaeognathae*, as all have cranial structures similar to that of *Struthio*, in which there is no actual mesokinetic joint. What is true for *Struthio* can possibly be regarded as being typical of the *Dromaeognathae*, excluding *Apteryx*, and of the *Crypturi* (e.g. *Crypturellus*) for reasons that will be discussed below.

The condition in *Struthio* is such that though kinesis is possible, it is exceedingly limited owing to anatomical features limiting the dorsal lifting of the

Plate. 4. Photograph of the adult skull to illustrate the m.orbitoquadratus.

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beak. The chief of these is the continuity of the septum, irrespective of its being partially or wholly ossified. The absence of a mesokinetic joint is secondary as it can only develop in birds when the trabeculo-capsular entity is interrupted by a fissura cranio-facialis. Because *Struthio* does not possess a fissura cranio-facialis, one would not expect to find a mesokinetic joint.

Another important anatomical pecularity is the m.orbitoquadratus (m.protractor quadrati). In *Struthio* this muscle is inserted onto the posterior face of the processus orbitoquadratus and is attached to the anterior surface of the basisphenoid and ventral part of the pleurosphenoid, ventral to the foramen profundum, and over the foramen prooticum, thus causing the truncus maxillo-mandibularis to penetrate the muscle. The distance between the processus orbitoquadratus and basisphenoid is small (Plate 5), however, and since the muscle fibres lie obliquely in the transverse plane their effective length is greater than it would be if they were horizontal (Plate 4).

For kinesis, that is, the dorsal lifting of the beak to be effective in the absence of a fissura cranio-facialis the dermal bones must be lifted off the mesethmoid

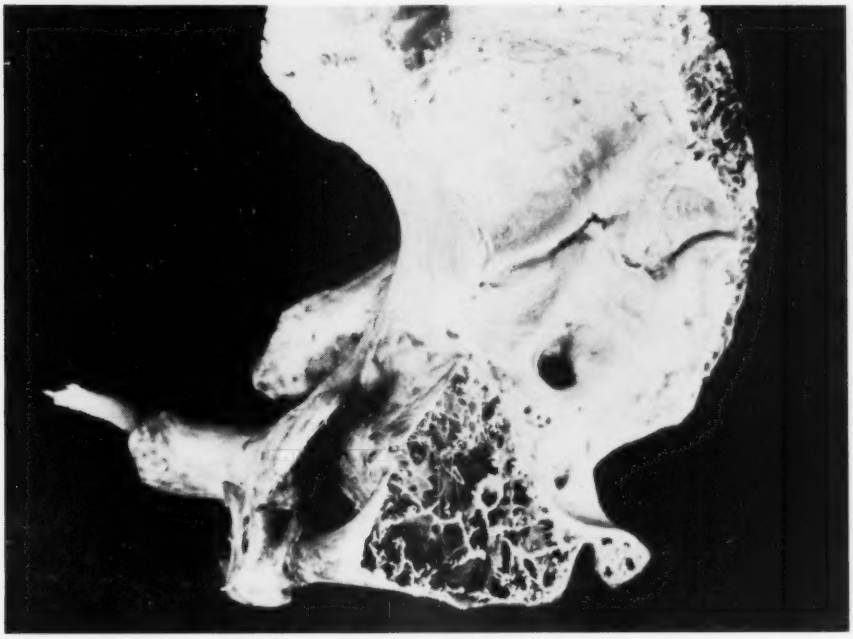

Plate. 5. Photograph of part of the adult skull illustrating the distance between the processus orbitoquadratus and basisphenoid.

(DE VILLIERS, 1946) as well as off the cartilaginous nasal septum. This is possible since the roofing bones are connected by loose connective tissue to the underlying nasal septum. For a lifting of the dermal bones to be possible there are two alternatives: either the relations of the dermal bones must permit sliding movements between opposing elements, or the bones must be of such a nature as to permit bending. The latter is the case in *Struthio*.

When the m.orbitoquadratus contracts, it pulls the processus orbitoquadratus backwards and upwards, thus moving its distal end forwards. Owing to the configuration of the articulatory head of the processus oticus and its relation to the squamosal (Plates 6 A and B), the quadrate, upon the contraction of the m.protractor quadrati, moves in a medial direction (Plates 6 A and B). Though the distance through which the quadrate moves is limited, it nevertheless causes a certain amount of dorsal lifting of the beak. The arthrosis between the quadrate and pterygoid is limited in extent. This is understandable because the limited amount of movement of the quadrate would be taken up by this synovial joint without effecting movement of the pterygoid. The other feature of the arthrosis is the structure of the posterior end of the pterygoid (Plate 7) which is vertical and flattened, thus forming a bony plate between the quadrate and processus basipterygoideus. The pterygoid is so constructed as to form a buttress

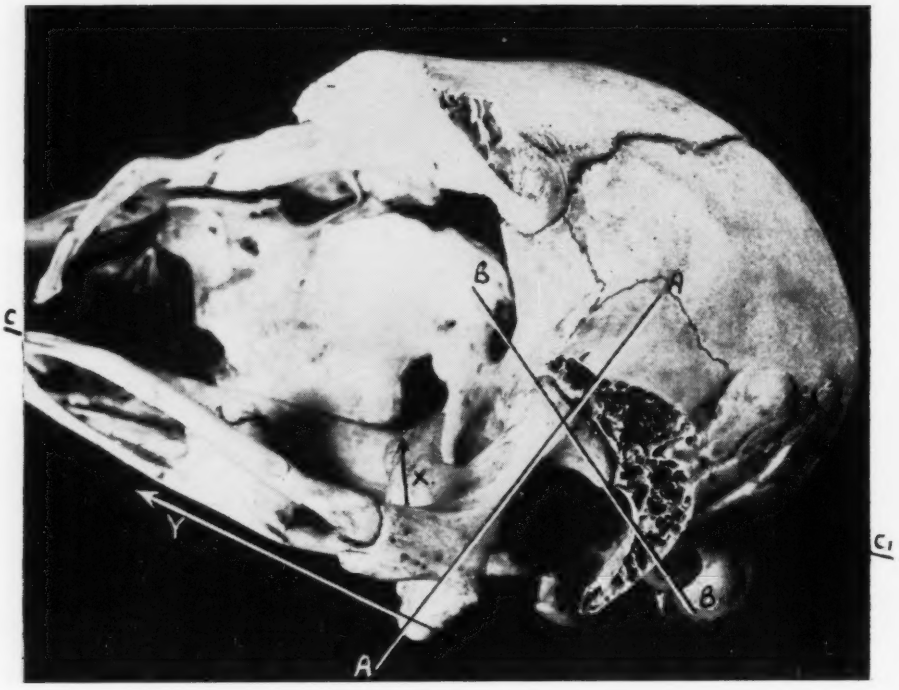

Plate 6 A. Photographs of the adult skull illustrating the relation of the processus oticus of the quadrate and the squamosal, and also to illustrate the directions of movement during kinesis. A) Postero-lateral aspect of adult skull with the processus zygomaticus of the squamosal removed to illustrate the processus oticus—squamosal arthrosis. A.A_1. vertical axis of quadrate; B.B_1. axis about which processus oticus can rotate; C.C_1. horizontal axis of skull; X. direction of movement of processus orbitoquadratus on contraction of the m.orbitoquadratus; Y. direction of force applied to pterygoid.

against the quadrate (Plate 7 and Fig. 34), enabling the pterygoid to be displaced forwards, thereby eliminating mutual movement between these two bones.

The movement between the pterygoid and processus basipterygoideus is in an anterior-posterior direction and the efficiency of such movement is assisted by a well-developed synovial cavity between the two bones.

The movement initiated by the quadrate drives the pterygoid forwards, thus drawing the rostral flanges of the pterygoid over the rostrum. These flanges are connected to the rostrum by loose connective tissue which does not impede their movement. This forward movement is transferred directly to the premaxillary by way of the palatine and maxillary. The shifting of the vomer is brought about by the maxillary and not directly by the pterygoid, as the nature of the connexion is such as to prevent any independent movement between the vomer and maxillary. The nature of the connexions and arthroses of the various elements inolved in kinesis is such that the maximal amount of

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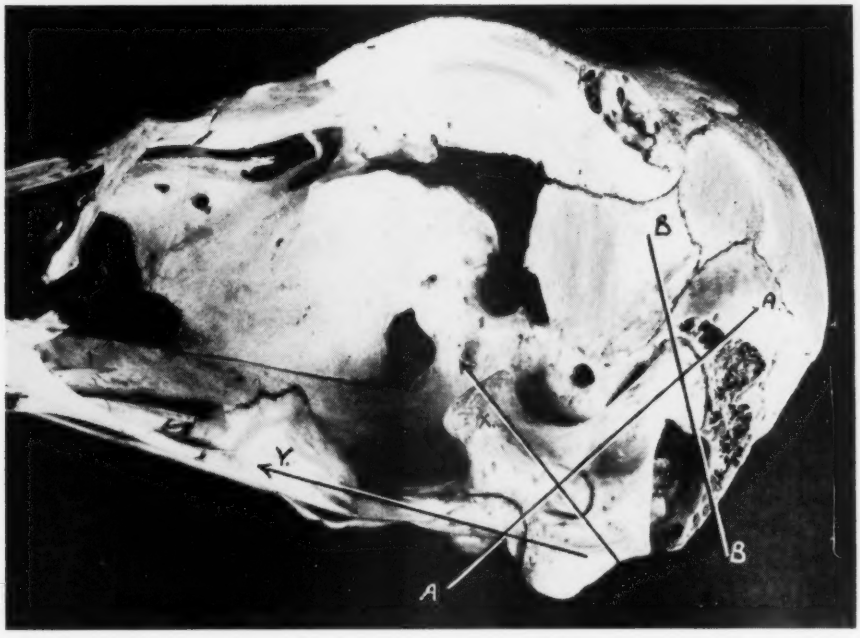

Plate 6 B. Antero-lateral aspect of adult skull.

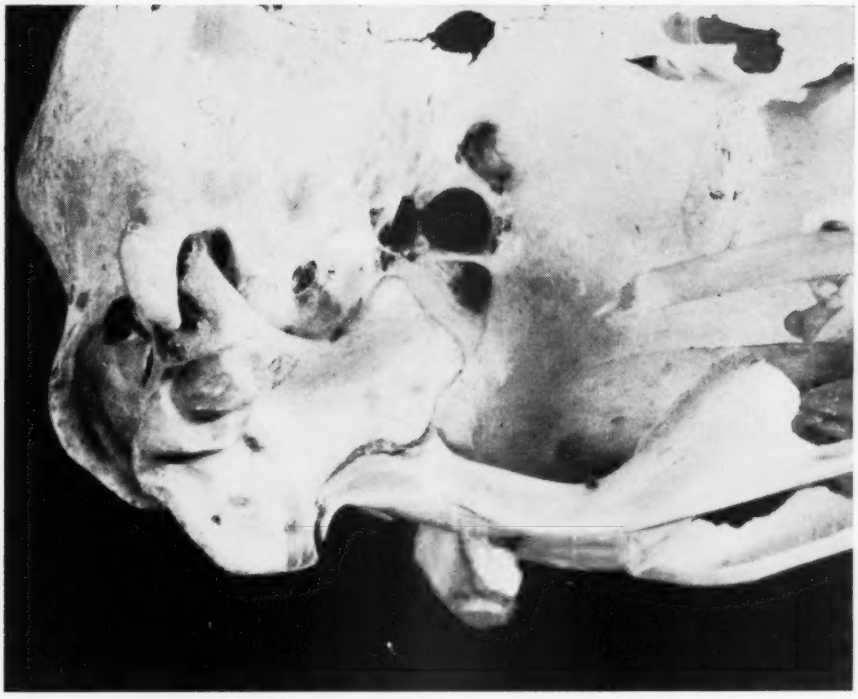

Plate 7. Photograph of part of the adult skull illustrating the nature of the arthrosis between the quadrate and pterygoid.

movement initiated by the quadrate is reflected in the premaxillary, without some of the movement being absorbed by loose connexions.

The degree to which the beak is displaced dorsally, owing to the forward movement of the palato-pterygo-quadrate bar, is limited, because of the absence of a true mesokinetic joint, which, of necessity, must be taken over by some other mechanism. Since no sliding movement is possible between the nasal process of the premaxillary and underlying nasal splints, it means that the anterior roofing elements must be lifted off the underlying septum. This lifting is made possible by the presence of loose connective tissue connecting the nasals with the nasal capsule beneath, and by the mesethmoid being loosely connected to the nasals and frontals.

Added to this limited lifting of the entire beak away from the septum, a certain amount of flexibility is found, not only in the nasal process of the premaxillary and nasals, but is also a condition of the laterally placed premaxillaries and maxillaries. If these elements were completely rigid, no dorsal lifting of the beak could be effected owing to the presence of the vomer as the limiting factor.

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As the vomer is trough-shaped between the palatal processes of the maxillaries and covers the rostrum ventrally, the space between it and the rostrum does not permit of its being lifted. De Villiers (1946, p. 35) states, quite correctly: "... that the mesokinesis must be restricted, for it is being constantly impeded by the presence of the vomerine trough below the septum." The nature of the connexion between the vomer and rostrum will, however, allow of a ventral shifting away from the rostrum, though this will only be very slight, only the posterior rim being depressed (de Villiers, 1946).

The lower temporal arch is composed of the jugal and quadratojugal rigidly connected through interdigitating processes. The posterior end of the quadratojugal fits into a lateral excavation of the quadrate, thereby preventing any movement between these two bones. The jugal interdigitates anteriorly with the maxillary thereby forming a rigid connexion. There is no diarthrosis at this point as in the case of extreme mesokinesis present in the order *Psittaciformes*, in which the temporal arch appears to be the chief levering apparatus for the snout (de Villiers, 1946). In *Struthio* this arch must be moved forwards with the movement of the quadrate, but only very slightly. The dorsal displacement of the arch can possibly be prevented by the processus orbitalis anterior of the lacrimal to which it is connected by ligamentous tissue. But this is not general because, if displacement is going to take place, it will be in the middle of the arch and not in the region of the lacrimal.

Of the *Dromaeognathae* excluding the tinamous, *Struthio* is possibly the most kinetic; *Apteryx*, being akinetic, representing the other extreme. This conclusion is based on the fact that in all forms other than *Struthio*, the anterior half of the palate is more complete because of the presence of palatal processes of the

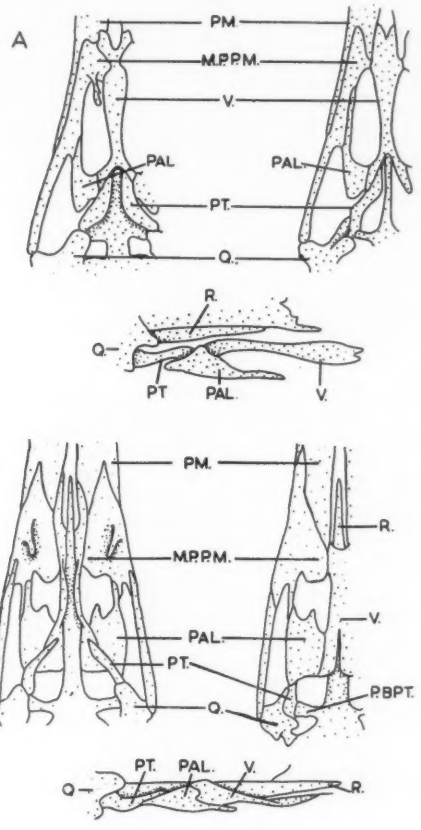

Fig. 35 A and B. Dorsal, ventral and lateral aspects of the palate of: A) *Dromiceius*. B) *Rhea*. C) *Casuarius*. D) *Rhynchotus*. All redrawn from McDowell (1948). *PAL.*, palatine; *P.M.*, premaxillary; *M.P.P.M.*, maxillopalatine process of the maxillary; *PT.*, pterygoid; *Q.*, quadrate; *R.*, rostrum; *V.*, vomer.

premaxillary. The degree to which the beak can be lifted depends, to a certain extent, on the flevibility of the premaxillary-maxillary bar. Because the palatal process of the premaxillary is broad, the degree to which it contacts the vomer anteriorly must, of necessity, stabilize the palate, thereby reducing kinesis to a minimum.

In forms such as *Casuarius* and *Dromiceius* the rigidity of the palate is obvious, as the premaxillary is produced into strong palatal processes which ankylose with the vomer, thus reducing the flexibility so essential for kinetic movement. However, this defect is overcome to a certain extent, by the bony oral roof lying wholly ventral to the rostrum which takes no part in its formation (McDowell, 1948) (Fig. 35 A and C) (cf. Fig. 35 B and D). The vomer therefore does not limit the dorsal lifting of the beak as much as it does in those forms in which it is closely applied to the overlying rostrum. The histological details of this area are not known, but there is possibly a limited amount of dorsal movement of the palate present.

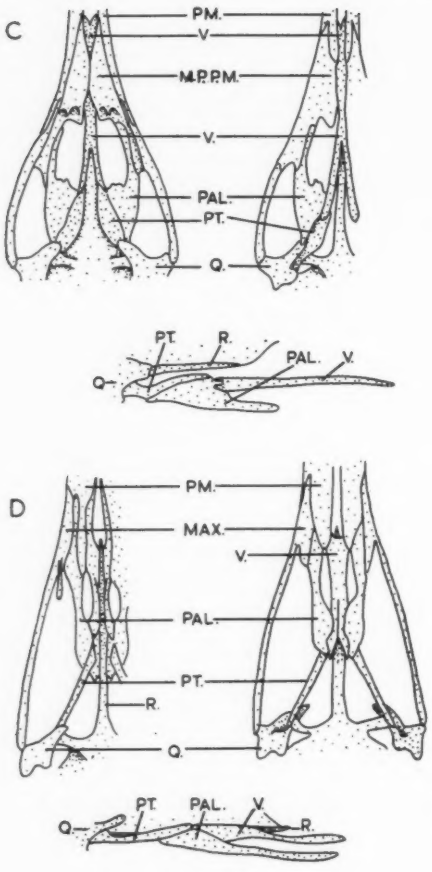

Fig. 35 C and D. Dorsal, ventral and lateral aspects of the palates of: C) *Casuarius*. D) *Rhynchotus*. *PAL.*, palatine; *P.M.*, premaxillary; *M.P.P.M.*, maxillopalatine process of the maxillary; *PT.*, pterygoid; *Q.*, quadrate; *R.*, rostrum; *V.*, vomer.

In *Apteryx, Crypturellus, Rhynchotus* and *Rhea,* kinesis is in all probability reduced to the absolute minimum or totally absent. This is to a certain extent confirmed by the findings of McDowell (1948). *Apteryx* and the tinamous are not grazing birds as are the others, and thus require a firm upper mandible. This is possibly neecessary for *Apteryx,* a bird of vermivorous feeding habits, using its long pointed beak as a means of digging (McDowell, 1948). In *Crypturellus* the rigidity of the palate is, to a certain extent, overcome by the presence of an extensive synovial cavity separating the vomer from the rostrum, thus permitting the vomer to be lifted against the rostrum. The tinamous feed partly on roots and require a strong pinching power (McDowell, 1948). How far kinesis is developed in these birds is unknown and further work will have to be done to solve this problem, when developmental material becomes available.

Rhea, Struthio, Casuarius and *Dromiceius* have become specialized toward an enlargement of the gape to premit the swallowing of large objects (McDowell,

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1948). Kinesis in *Struthio,* as has been shown, is limited; in *Rhea, Casuarius* and *Dromiceius* the structure of the skull is such that kinesis must be even more limited, because of the stable palate anteriorly, and the close proximity of the vomer to the rostrum. It is reported that *Rhea* is proficient in fly-catching, and McDowell (1948) suggests a correlation between a wider gape and this habit, though *Rhea* cannot be sufficiently kinetic to permit a wider gape.

Finally, as we have seen, *Struthio* possesses in the embryo, a connexion between the premaxillary and the vomer which is lost after hatching. It has been suggested that this is to stabilize the embryonic palate as the thickness of the shell must present a problem to the chick attempting to peck its way out. When doing so it must jar the palate, and so sturdier and more massive bones are found in the *Ratitae* than in the *Neognathae,* with the tinamous having thin bones constituting the palate, as the shell is thin (McDowell, 1948). In *Struthio* the stability of the palate in the embryo must exist for this mechanical reason. Only after the chick has emerged, does the premaxillary lose its connexion with the vomer, while in the other forms this connexion is retained, thus supporting the contention that the *Dromaeognathae,* excluding *Struthio,* are neotenic as far as the feature under discussion is concerned.

GROUNDS ON WHICH DROMAEOGNATHAE CAN BE REGARDED AS NEOTENIC

(1) Non-fenestrated septum.
(2) Presence of premaxillary-vomer connexion in all excluding *Struthio*; the latter has this arthrosis in the embryo.
(3) Failure of the cranial sutures to close (McDowell, 1948) i. e. the bones are not in synostosis.
(4) Failure of anterior bony nares to close behind and become separated from preorbital fossa (McDowell, 1948).
(5) Imperfect fusion of vomerine halves in *Rhea, Rhynchotus, Casuarius, Apteryx* and *Dromiceius* (McDowell, 1948).
(6) The vomero-pterygoid connexion in the adult *Dromaeognathae,* excluding *Struthio,* an embryonic character of carinate birds (Pycraft, 1900).
(7) Failure of wings to develop beyond the embryonic stage of carinate birds.
(8) Structure of feathers.
(9) The large size is not infrequently an indication of neoteny (de Villiers, 1946).

M. WEBB

HYPOTHESES CONCERNING THE PHYLOGENY OF WALKING BIRDS

The hypotheses to be discussed here are put forward with a certain amount of reservation. The most important limiting factors are the lack of embryological data of the *Dromaeognathae* and the difficulty experienced in obtaining adult skulls of the more important members of the group. The result has been that a comparison of the members within the group is based, for the greater part, on previous works figuring these skulls, which differ from one to another in some of the fundamental features. Despite these difficulties, it is felt that some conclusions can be drawn, which will either be confirmed or rejected as additional information becomes available.

It is important to remember that the basic classification of birds is based on the structure and relations of the palatal bones, though with further knowledge forthcoming this may have to be modified accordingly. The substance of the two hypotheses to be discussed here, applies especially to the palate. Previous workers who have used the skulls as a means of determining the phylogeny of the group have used other cranial features together with the structure of the palate, and as a result some of the more important features tend to become oscured. The nature of the adult palate, as well as its structure in the embryonic series, and the relations of the bones in the earlier and later stages, has already been dealt with. From the information available it is clear that *Struthio* possesses a palate from which that of the other *Dromaeognathae* can be derived.

The region of greatest weakness and stress, in the palate of *Struthio,* is obviously that of the palatine, which is a relatively thin connecting rod between the pterygoid and maxillary. This rod is strengthened by the outer process of the maxillary lateral to the palatine, and by the ligament extending from the medio-dorsal surface of the palatine to the inner process of the maxillary (Fig. 36 and Plate 1).

From the nature of these supports, in relation to the palatine, it is obvious that they are there for a very definite purpose, that of strengthening the palatine, thus preventing it from flexing inwards or outwards. It may therefore be inferred that an ancestral form similar to *Struthio* possibly lacked these supports. It is possible that the *Dromaeognathae* may have been derived from such an ancestral form.

Fig. 36 is a graphic representation in support of the following argument. As has already been seen, the pterygoid is shifted forwards upon the contraction of the m.protractor quadrati, and the direction of movement is parallel with the rostrum; there is no independent movement between any of the bones constituting the palate. The force then applied to the pterygoid is transferred

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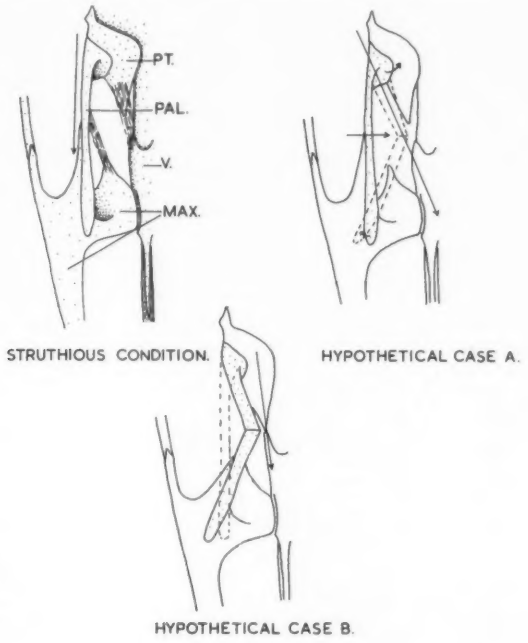

Fig. 36. Diagramatic representation of the inward flexure of the palato-pterygo-quadrate bar in *Struthio*. *MAX.*, maxillary; *PAL.*, palatine; *PT.*, pterygoid; *V.*, vomer.

to the palatine resulting in considerable stress being exerted on it in an effort to displace the maxillary which will bring about the lifting of the beak. Not only is the maxillary concerned in this process, but it also indirectly shifts the vomer anteriorly, thus increasing the stress applied to the palatine in an effort to perform these functions. To prevent the palatine buckling, the maxillary supports were developed, both laterally and medially, resulting in the condition found in *Struthio* today. If the ancestral form had a palate identical with that of *Struthio* (excluding the palatal supports) the stress applied to the palatine would have been too great, thus causing an inward or outward flexure. According to our hypothesis a flexure did take place in an ancestral form and it was of course, inwards.

As a result of this flexure, the pterygoid was drawn slightly forwards, thus making contact with the vomer. Such contact is essential if the presumably already limited kinesis was not to be further reduced, since the movement of the palatine would no longer be forwards and parallel to the rostrum, but diagonally across the face of the palate (Fig. 36). To overcome this deficiency the pterygoids touched the vomer posteriorly, thereby establishing a means whereby the beak could be dorsally lifted, not directly through the palatine but through the vomer. Since the vomer is now moved directly by the pterygoid, the vomer-maxillary connexion is lost and a new connexion develops between the vomer and premaxillary which is actually present in the *Dromaeognathae*, although

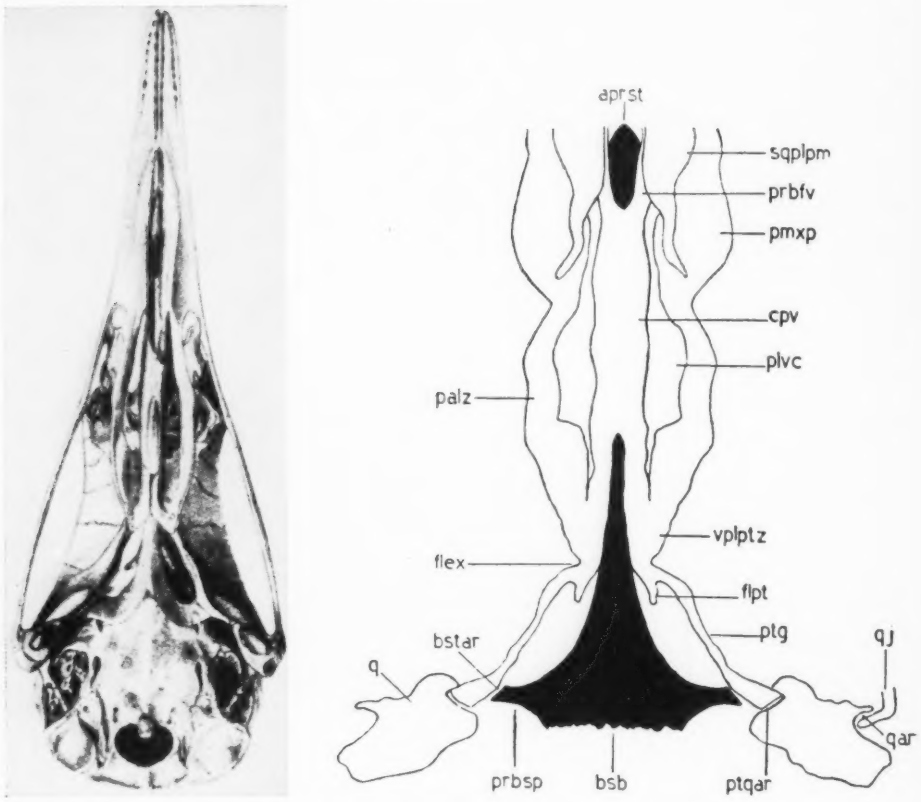

Plate 8 A. Ventral view of *T.major robustus*. (After W. K.. Parker, 1866).

Plate 8 B. Graphical reconstruction of the osseous support of the palatal roof of *Crypturellus*. (After de Villiers, 1946).

in *Struthio,* confined to the embryo. An explanation of this phenomenon will be given later.

The question that now arises is: is this assumption supported by the palates of the other *Dromaeognathae?* Plates 8, 9 and 10 figure the palates of several of these, and they all show a certain degree of flexure of the palato-pterygo-quadrate bar. It is minnmal in *Apteryx* and maximal in *Crypturellus.* This flexure of the palato-pterygo-quadrate bar has not only made it possible for the vomer and pterygoid to meet, but also for the palatine to meet the vomer, which, however, still separates the palatines from each other. In the *Neognathae* the palatines are not separated by the vomer medially, but they meet in the middle line (PYCRAFT, 1900). The palates of the *Dromaeognathae* vary in detail, but they all conform to a ground plan which can possibly be derived from the modified struthious palate.

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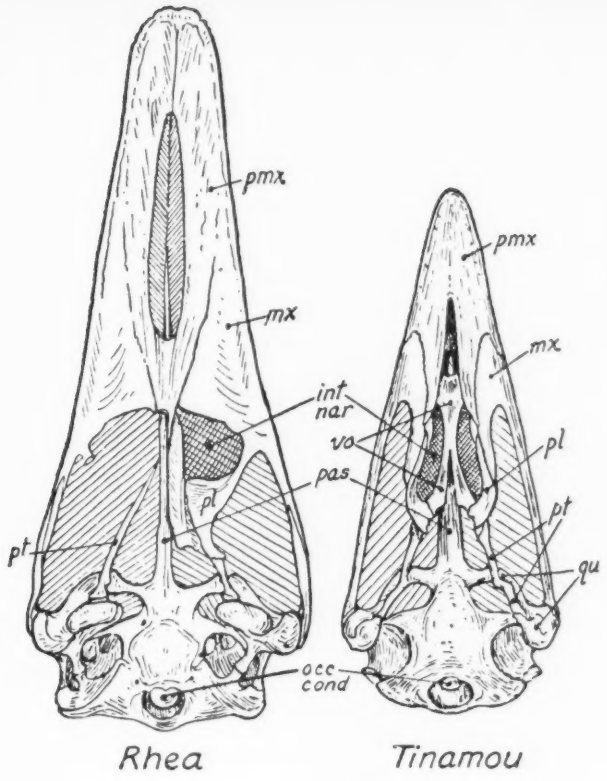

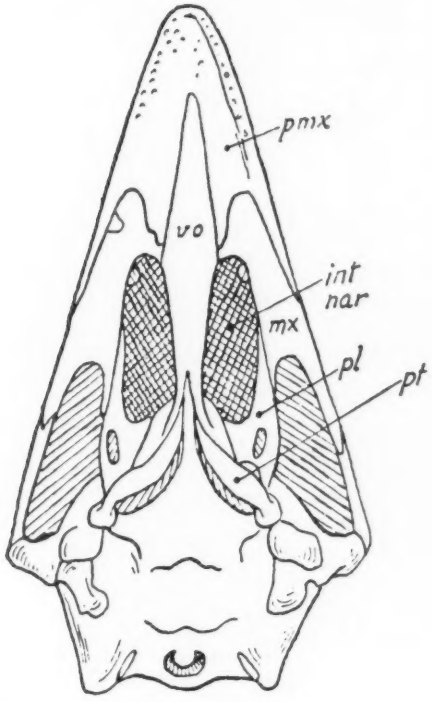

Plate 9. The palates of some *Dromaeognathae Rhea* (after Gregory, 1951), *Tinamou* (after Pycraft and Gregory); Emu (after Pycraft).

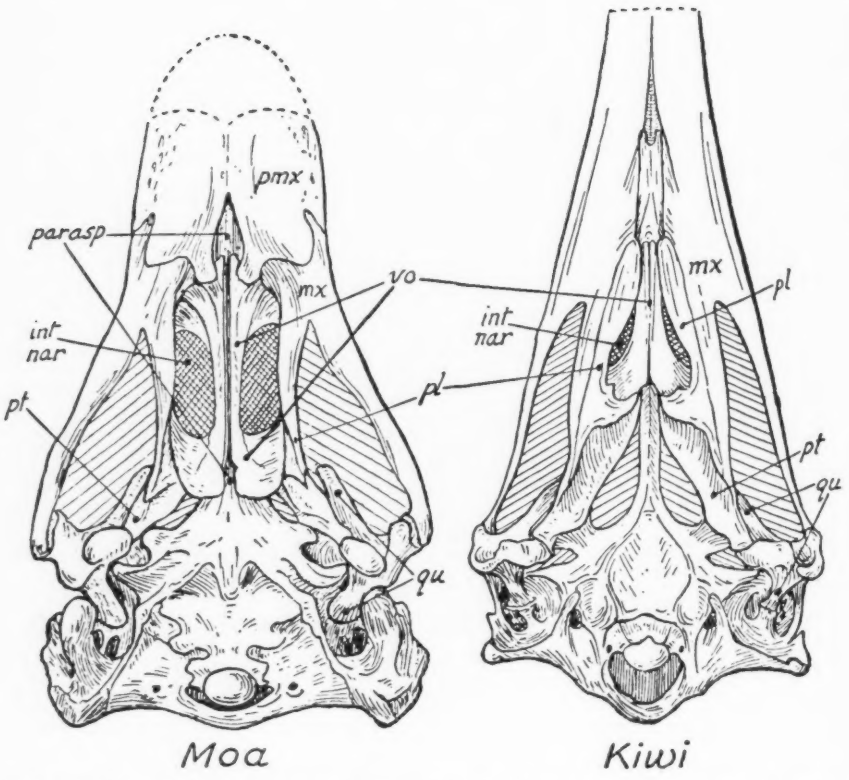

Plate 10. The palates of two other *Dromaeognathae*. Moa (after Pycraft and Gregory); Kiwi (after Gregory).

Of the *Dromaeognathae*, excluding *Struthio*, there is a single common feature: the vomer touches both palatine and pterygoid (McDowell, 1948). Huxley (1867) was of the same opinion except that he considered *Dinornis* and *Struthio* to be similar on this particular point. Fig. 35 figures the palates of the *Dromaeognathae* as drawn by McDowell (1948). These figures differ to a certain extent from the illustrations of the palates in Plates 8, 9 and 10. In *Rhea* the palato-pterygo-quadrate bar is as straight as it is in *Struthio* and *Apteryx*, while in *Dromiceius*, *Casuarius* and *Rhynchotus* there is a progressive increase in the inward flexure. The condition in *Rhea* has possibly been brought about by excessive or differential growth of the medial margin of the palatine and the postero-lateral flanges of the vomer, while the pterygoids are reduced to rods. In *Dromiceius* and *Casuarius* the pterygoids are longer and more rod-like than in *Struthio*, but nevertheless possess distinct rostral flanges, which are not as pronounced as in *Struthio*, and the flanges do not reach the rostrum.

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The palatine in *Dromiceius* and *Casuarius* has palatal processes meeting the vomer posteriorly which also possesses pronounced posterior processes.

The fundamental difference between the palates figured on Plates 8, 9, and 10, and the palates as drawn by McDowell (1948) is that in the one series there is an increase in the inward flexure of the palato-pterygo-quadrate bar, being minimal in *Apteryx* and maximal in *Crypturellus,* while in the other series (McDowell, 1948), *Rhea, Struthio* and *Apteryx* have straight palato-pterygo-quadrate bars, and only *Dromiceius, Casuarius* and *Rhynchotus* show flexion of the palato-pterygo-quadrate bar. In considering this latter condition it would appear that there have been two distinct lines of evolution, the one line including such members as *Rhea, Struthio* and *Apteryx,* and the other line including *Dromiceius, Casuarius* and *Rhynchotus.*

In spite of these two distinct groups, the hypothesis already given can be applied here with certain modifications. Still assuming that the *Dromaeognathae* arose from a bird with a palate similar to that of *Struthio* without the palatal supports; instead of flexion of the palato-pterygo-quadrate bar taking place as a means of stabilizing the palate in all the forms, differential growth of the palatine took place resulting in the condition in *Rhea* and *Apteryx,* while in *Dromiceius, Casuarius* and *Rhynchotus* flexure did take place. Whichever course was followed, and at the same time assuming that *Struthio* is the most primitive of the *Dromaeognathae,* in respect of the palate, from which the palates of the other *Dromaeognathae* can be derived, it does not mean that *Struthio* is primitive in respect of the *Neognathae* as will presently be shown.

From the foregoing argument it may be inferred that *Struthio* shows a character not present in any other dromaeognath and must therefore be regarded as being in no way related to the other *Dromaeognathae.* This character is the absence of the vomer-pterygoid connexion, so characteristic of the *Dromaeognathae* as a whole.

It may, however, be assumed that the struthious condition is primary since contact between the vomer and pterygoid is prevented by the straightness of the palato-pterygo-quadrate bar. On the other hand the condition of the palate, and more particularly the vomer, in *Struthio* may be considered as primitive. Pycraft (1900), however, considers that the vomer in *Struthio* is degenerate, having lost its connexion with the pterygoid. This suggestion is supported by McDowell (1948) who regards the ligament connecting the vomer and the pterygoid as representing the posterior part of the vomer, which has become lost. Whatever the case may be, it is obvious that *Struthio* is isolated and not closely related to the other *Dromaeognathae.*

This brings us to an exceedingly difficult question: the inter-relationships of the various members of the group. *Struthio* is definitely isolated, while the other members of the group appear to be more closely related. The relation of the vomer to the maxillary is retained in all the members of the *Dromaeog-*

nathae to a greater or lesser degree, while the relation of the vomer to the premaxillary is of greater interest, because in the adults of all the *Dromaeognathae*, excluding *Struthio*, there is a connexion between these two elements. This connexion is present in the embryo of *Struthio*, but is lost in the adult. If *Struthio* is considered as the most primitive member of the group, this factor alone illustrates the neoteny of the other *Dromaeognathae*. If the majority of the group are neotenic in the possession of this one feature, is it not possible for *Struthio* to be neotenic too? If this be the case the whole group would be neotenic.

This idea of neoteny is not new, as it was first suggested by DE BEER (1937 and 1940); LOWE (1942), and then by DE VILLIERS (1946) and MCDOWELL (1948). DE VILLIERS (1946) qualifies this idea of neoteny by stating that it is difficult to distinguish between neotenic and truly primitive characters and defines neoteny (1934, p. 410) as follows: "If occasionally a member of a group in the adult retains organs in a condition characteristic of the ontogenetic condition of other members of the group, or if in the anatomy of the said member mutual relations of organs are such as to prevail in the ontogeny of other members of the group, the said member displays neotenic features."

From this it may be said that the condition of the anterior palate in the *Dromaeognathae*, excluding *Struthio*, is normal and that *Struthio* is specialized by not possessing the premaxillary-vomer connexion in the adult. But, for reasons already mentioned, *Struthio* is regarded as a primitive dromaeognath, while the other members of the group are neotenic.

LOWE (1942) mentions on p. 18: "It is for those holding the view that the Struthiones are the degenerate descendants of a once volant protocarinate stock to explain why this striking morphological character has come to pass...", and further: "... submitting the following points for the explanation if the phenomenon of neoteny is to be accepted, as the cause of the present morphological conditions in the struthiones." It is quite obvious that Lowe was toying with the idea of neoteny, though he repeatedly rejected the theory of the neotenic origin of the *Ratitae* (FRANK, 1954). In 1928 on page 233, Lowe makes the following statement: "... we have in the palatine morphology of the struthious *adult* the same phase of evolution as is obvious in the *embryos* of the *Neognathae*," and in the next paragraph, states: "The only possible conclusion to be drawn, therefore, is that the struthious palate is more primitive than the neognathous and that in it we have an accurate presentment of what the early avian palate was like." These statements can be interpreted in no other way than that Lowe was quite aware of the phenomenon of neoteny, but was not aware of the significance of it in respect of the origin of the group.

MCDOWELL (1948) in his "hypothesis of reversal" considers that the palaeognaths are a reversal from a neognathous stock or stocks, and supports this contention with the similarity shown in the palates of the *Rheidae* and *Tinamidae*.

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These similarities are also borne out in the nasals, lacrimals, calvarium, costal processes and other structures resulting in what he considers a close phylogenetic relationship. McDowell (1948) also considers that the *Ratitae* have evolved from flying forms, and that *Rhea* evolved from the *Tinamidae* despite its having a more primitive palate.

The "primitive" palate of *Rhea* can be explained by neoteny, and for that matter the explanation can also apply to the other dromaeognaths. McDowell (1948) gives examples of neoteny in the palaeognaths, for example the failure of the anterior bony nares to close behind, thus separating the preorbital fossa. This cannot be regarded as reptilian, as in the *Archaeopterygidae* (Heilmann, 1926) the nares and preorbital fossae are separated; this is also true of the *Pseudosuchia*.

There are other neotenic features of the dromaeognaths, such as the uninterrupted septum, which in the adult carinates is interrupted by the fissura craniofacialis; imperfect fusion of the vomerine halves in *Rhea, Rhynchotus, Casuarius, Apteryx* and *Dromiceius* (McDowell, 1948); the failure of the cranial sutures to undergo synostosis. Then there are neotenic characters more specific for some members of the group. In the *Carinatae* there is a vomero-pterygoid connexion in the embryo only; but this occurs in all dromaeognaths excluding *Struthio*. The failure of the wings to develop beyond the embryonic stage of carinate birds, and the structure of the feathers can also be regarded as being neotenic.

Since there appears to be considerable evidence in favour of regarding the dromaeognaths as neotenic, they can also be regarded as being primitive, as the distinction between primitive and neotenic characters is difficult to determine. This conception of primitiveness does not apply to what Huxley (1867) and Pycraft (1900) had in mind when they considered the *Dromaeognathae* as primitive. Both these authors regarded them as primitive, because they show an ascending degree of palatal complexity, ending in the tinamous, which are regarded as the "link" between the so-called flying and walking birds. In other words, Huxley and Pycraft consider the *Dromaeognathae* as primitive in comparision with *Carinatae;* also that the dromaeognaths were derived from some ancestral form that was fully capable of flight. The results of the present investigation tend to confirm these views. As a result of neoteny they show definite primitive characters, especially in regard to palatal structures, and have also become highly specialized.

From the above, it should be clear that the previous views held in considering the *Dromaeognathae* as the most primitive of all birds is not supported, but rather that they are a neotenic off-shoot of some ancestral carinate bird or birds. The question that now arises is: which member of the *Dromaeognathae* can be regarded as the most primitive? The answer to this question must, of necessity, await further knowledge of the ontogeny of the other members. In

this paper *Struthio* is considered as filling this position, because it is possible to derive the palates of the other *Dromaeognathae* from one similar to that of *Struthio;* and that *Struthio* displays a condition of the palate in the adult similar to that of the carinate birds in not having a vomer-pterygoid connexion, and lastly the tendency for the *Dromaeognathae* to become akinetic.

From the latter statement it would be assumed that the *Dromaeognathae* were derived from some kinetic form that was carinate. This means, therefore, that the ancestral bird was kinetic, but phylogenetically no one has shown that birds were derived from a kinetic ancestor. Forms such as *Archaeornis* and *Archaeopteryx* of the upper jurassic, or *Hesperornis,* or *Ichthyornis* of the upper cretaceous have been shown to be kinetic. Going even further back, no one has shown that the *Pseudosuchia,* and in particular *Euparkeria,* were kinetic.

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Though it would be unwise at this stage to attempt to give a definite diagram on the relations of the various groups, Fig. 37 is a phylogenetic diagram of the *Dromaeognathae* based on the classification of McDowell (1948) and according to the theory that the *Dromaeognathae* are neotenic. McDowell (1948) has gone into considerable detail concerning the palaeognaths and has come to the conclusion that on morphological grounds alone they cannot be defined, but that there are four main lines. He does not suggest any definite relations between these lines and the *Neognathae* because of the lack of fossil evidence. To this can be added the lack of ontogenetic data.

THE LOWER JAW IN BIRDS

The investing bones of lower jaw of birds are, for the greater part, numerically constant, though the terminology may vary. There are five such bones, namely dentary, surangular, splenial, angular, and gonial, the last having by some authors, been referred to as the complementary, while the splenial has been given the alternative name of opercular. The articular is an ossification of the pars articularis of Meckel's cartilage. The anterior end of Meckel's cartilage is either absorbed or ossified forming the mento-mandibular, alternatively known as the processus longus mallei (Gaupp, 1913) which Stresemann (1927—1934) has reported for some *Accipitres.*

Of all the birds so far investigated, only *Gallus* has four dermal bones, the gonial being absent (Parker, 1866a) and (Tonkoff, 1900). As a result Voit (1924) together with Parker (1866a) and Tonkoff (1900) suggested that the gallinaceous birds can be characterised by the absence of the gonial. However, Magnus (1870) made no mention of the loss of the gonial in birds.

Parker (1891) describes five dermal elements in *Apteryx* and names the medial proximal element the coronary. This element occupies the same topo-

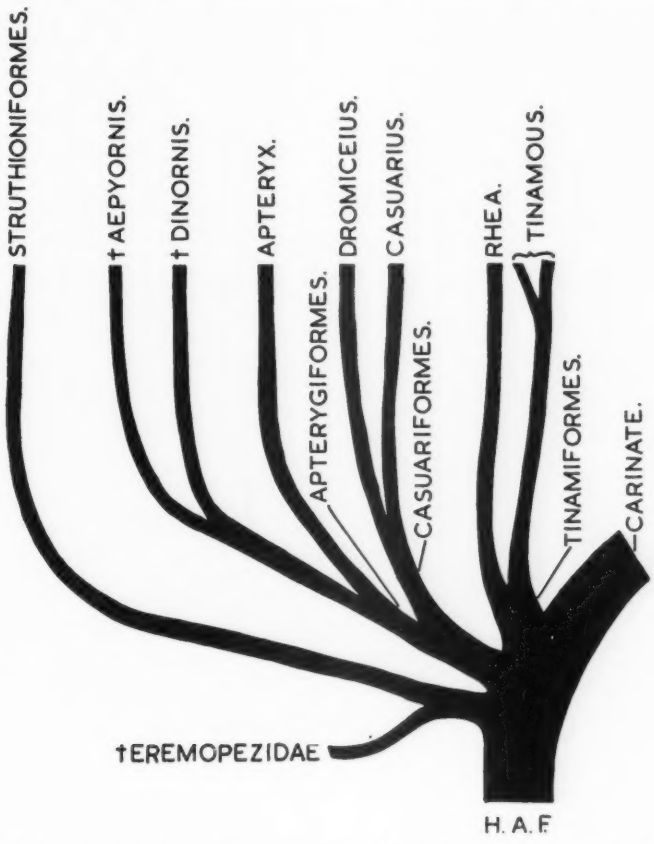
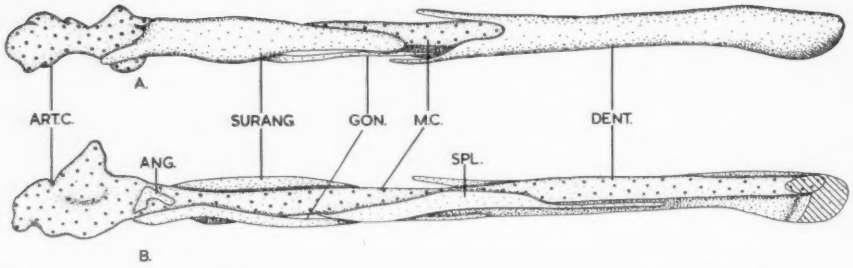


Fig. 37. Phylogenetic tree of the *Dromaeognathae* based on the classification of McDowell (1948) and according to the theory that they are neotenic. *H.A.F.*, hypothetical ancestral flying form rather like a member of the tinamous.

graphical position as the gonial and must therefore be considered as the gonial. *Struthio* and *Dromiceius* also have five dermal elements. PARKER (1866) identified the medial proximal element as the coronoid which, however, will be shown to be the gonial.

VOIT (1924) has gone into considerable detail concerning the homology of the gonial, and has proved conclusively that it is homologous with the so-called complementary of CUVIER, GAUPP (1913), SUSCHKIN (1899), and MAGNUS (1870), and with the coronoideum of PARKER (1869) and OWEN, GAUPP (1913) showed that the coronoideum of PARKER (1869) and OWEN is homologous with the complementary.



Figs. 38 A and B. Graphic reconstruction of the lower jaw of the 15-day old embryo. × 5. A) Lateral aspect. B) Medial aspect. *ANG.*, angular; *ART.C.*, articular cartilage; *DENT.*, dentary; *GON.*, gonial; *M.C.*, Meckel's cartilage; *SPL.*, splenial; *SURANG.*, surangular.

THE LOWER JAW IN STRUTHIO

(a) Ontogeny

Meckel's cartilage is laid down early in embryonic development, being present in the embryo of 9.2 mm. and is closely associated with the pars quadrata (FRANK, 1954, Fig. 2). It rapidly extends anteriorly and by the 15-day stage it nearly reaches to the end of the upper jaw. During this process mesenchyme condensations have been arranging themselvers round the cartilage. Osteoblasts are differentiated, and by the laying down of collagen fibrillae, will give rise to the bony elements constituting the lower jaw. These centres of ossification constitute the anlagen of the five dermal bones of the lower jaw.

The actual age at which these dermal elements are laid down is difficult to determine, but it takes place between the 14th and 15th day stage. In the former the mesenchymatous condensations are present without the presence of osteoblasts, while in the latter all five elements are present (Fig. 38 A and B).

The dentary in the very earliest discernible anlagen is unpaired. This condition is undoubtedly secondary as this bone is fundamentally a paired structure. The anlagen has two posteriorly directed mesenchymatous condensations lying lateral to Meckel's cartilage. By the 15th day stage there is considerable ossification of the dentary which completely covers the antero-lateral surface of the Meckelian cartilage and terminates posteriorly as a dorsal and ventral process (Fig. 38 A). The only other completely lateral bone is the surangular which is posteriorly situated and extends back as far as the articular cartilage, and anteriorly as far as the two posteriorly directed processes of the dentary. This element is pierced by a foramen for the passage of the ramus mandibularis externus, a sensory branch of the r.mandibularis of V.

The other three dermal elements of the lower jaw are medial and ventral in position. The splenial is the anterior medial bone of the three; in the 15-day stage it is splintlike and covers the cartilage of Meckel in the middle (Fig. 38 B).

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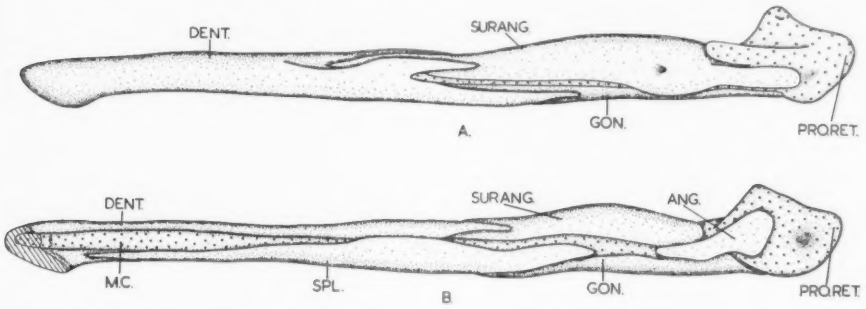

Figs. 39 A and B. Graphic reconstruction of the lower jaw in the 27-day old embryo. × 5. A) Lateral aspect. B) Medial aspect. *ANG.*, angualr; *DENT.*, dentary; *GON.*, gonial; *M.C.*, Meckel's cartilage; *PRO.RET.*, processus retroarticularis; *SPL.*, splenial; *SURANG.*, surangular.

The next bone is the gonial, which is also splint-like and lies ventral to the cartilage of Meckel, extending from the articular cartilage to the ventral process of the dentary (Fig. 38 A and B). Last of the dermal bones is the angular, which is small and "L"-shaped, situated antero-medially to the articular cartilage (Fig. 38 B).

In the 27-day old embryo there is an increase in the complexity of the membrane bones (Fig. 39 A and B). Posteriorly the dentary has become divided into three well-defined process; dorso-medial, dorso-lateral and ventro-lateral. Between the former two processes fits the anterior extremity of the surangular, and becomes syndesmotically connected to the two processes of the dentary. The surangular has increased considerably in size by a process of appositional growth and extends far back on the lateral surface of the articular cartilage (Fig. 39 A).

The splenial constitutes the main medial element of the lower jaw and extends practically from the anterior tip to a short distance in front of the gonial (Fig. 39 B). The gonial forms the ventral covering to the cartilaginous rod and extends back to the articular cartilage. The angular remains small in comparison with the other bones, but has now lost its "L"-shape and covers part of the medial surface of the articular cartilage posteriorly, as well as a small portion of Meckel's cartilage (Fig. 39 B).

Little change has taken place by the 34-day stage, except for an increase in the size of the dermal components with a corresponding closer relation between them. Meckel's cartilage has become reduced in diameter. Even in the 37-day stage it persists but shows further reduction in size. The articular cartilage up to the 37-day stage shows no signs of ossification. All the elements of the lower jaw are now in syndesmosis, which gives strength and stability to the whole structure, which would otherwise be rather fragile, considering the nature of the bones, which are for the greater part, thin and rod-like.

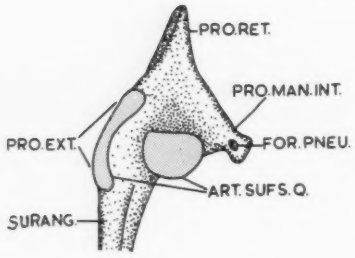

Fig. 40. Sketch of the articular in the adult, dorsal aspect. *ART.SUFS.Q.* articulatory surfaces for quadrate; *FOR.PNEU.*, foramen pneumaticum; *PRO.EXT.*, processus externus; *PRO.MAN.INT.*, processus mandibularis internus; *PRO.RET.*, processus retroarticularis; *SURANG.*, surangular.

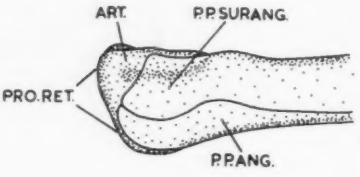

Fig. 41. Sketch of the pars articularis of the adult lower jaw. Lateral aspect. *ART.*, articular; *P.P.ANG.*, posterior process of angular; *P.P.SURANG.*, posterior process of surangular; *PRO.RET.*, processus retroarticularis.

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(b) Adult

The condition in the adult is fairly constant. In all skulls studied there is a foramen approximately halfway along the length of the lower jaw, bounded in all cases by the two lateral processes of the dentary and the surangular thus forming the so-called foramen mandibulae anterius.

The proximal portion of Meckel's cartilage ossifies as the articular, which is divided into very distinct regions: processus internus, p.externus and p.posterior (p.retroarticularis). Medially there is a fossa for the insertion of the m.depressor mandibulae. The dorsal surface of the articular has two articulatory facets for the quadrate. The outer facet is directed antero-posteriorly and is situated on the p.externus; the inner facet is located in the depression formed between the p.internus and p.externus (Fig. 40).

The proximal elements are for the greater part synostotically connected with the articular; but in the majority of the adult specimens studied the angular and surangular retain their identities, though they are not very clearly demarcated (Fig. 41 and Plate 11). The gonial, however, is always synostotically fused with the articular. The splenial overlaps the anterior process of the gonial posteriorly, and extends anteriorly as a thin rod of bone on the medio-ventral margin of the dentary to all but meet in the middle line.

Discussion

The lower jaw of *Struthio* agrees in all fundamental respects with that of the other *Dromaeognathae* as well as with that of the *Carinatae*. The jaw is divided into three regions, the pars anterior constituting that part in which the two halves are united; the pars posterior, being all that region posterior to the pars

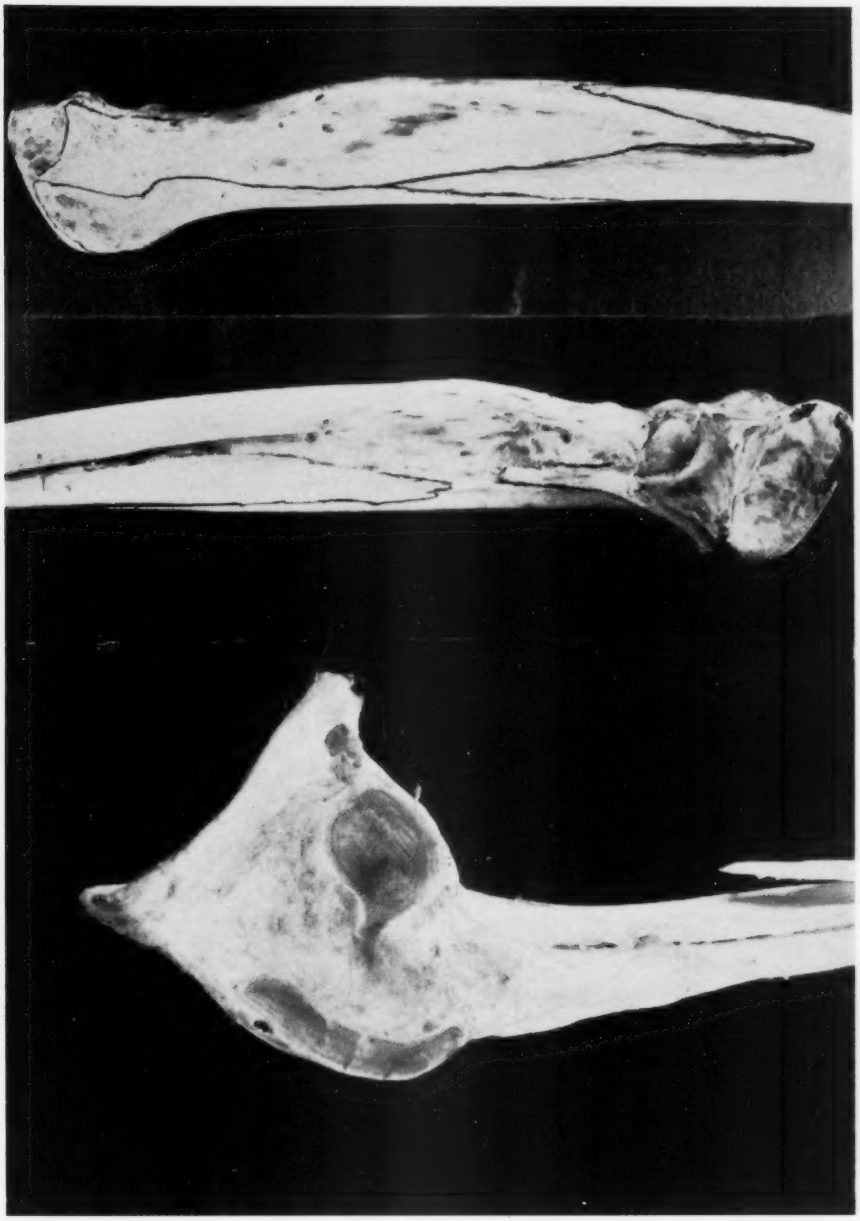

Plate. 11. Photographs of part of the adult's lower jaw. Lateral, medial and dorsal.

anterior. The pars posterior terminates in a sub-division, the pars articularis, which is concerned with the articulation with the quadrate (STRESEMANN, 1927—1934).

The anterior two-thirds of Meckel's cartilage becomes absorbed soon after hatching (PARKER, 1866). In some *Accipitres* the Meckelian cartilage ossifies in two centres, a distal centre, the mentomandibular, and a proximal centre, the articular, while in other birds there is only one ossification centre, the articular (STRESEMANN, 1927—1934).

The number of dermal bones in the lower jaw is five, having been reduced from the typical seven found in the *Sauropsida.* The coronoid and post-splenial have been lost. The latter element is only known in *Seymouria;* the gonial becomes fused with the articular early in the ontogeny and seldom appears as an independent element (VERSLUIJS, 1924). VERSLUIJS names the bones of the lower jaw the dentary, coronoid, angular, gonial and splenial, thereby not recognizing a supra-angular (surangular).

STRESEMANN (1927—1934) is not prepared to state definitely whether the postero-lateral bone lying between the dentary and articular is the coronoid or the surangular. The upper edge of this element serves for the attachment of the m.adductor mandibulae externus.

The small bone on the inner surface of the supraangular (surangular) is the gonial, according to VOIT (1924). It is the complementary of STRESEMANN (1927—1934) and MARINELLI (1936), while VERSLUIJS (1924) regards the complementary as the coronoid. STRESEMANN (1927—1934) states that the complementary is absent in *Struthio,* but this is not true, as a small element does appear on the inner surface of the surangular, and becomes synostotically fused with the articular. It is absent in *Sphenisci, Striges, Columbae* and *Gallus* (STRESEMANN, 1927—1934).

The postero-lateral element, here referred to as the surangular, is laid down as one bone, and must, therefore, be either the coronoid, or surangular. In reptiles both are present, the coronoid being the anterior of the two and raised up into a pronounced process; the processus coronoideus. STRESEMANN (1927—1934) figures the lower jaw of *Crax alector* after LEBEDINSKY (1920) as possessing a processus coronoideus, however not indicating whether it is a process of the surangular or a separate element. It can be stated with certainty that the postero-lateral bone in *Struthio,* and in birds in general, is the surangular, as the coronoid in reptiles does not extend back to the articular, and is situated anteriorly to the surangular; moreover, there is no pronounced dorsally projecting process, so characteristic of the coronoid.

In the adult *Crypturellus* the lower jaw was badly damaged during microtomization; the posterior end does, however, indicate the presence of a gonial which, together with the surangular and angular, become synostotically fused

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with the articular. The extent to which it extends back cannot be determined. The anterior end of Meckel's cartilage is apparently not ossified.

A microtomized head of adult *Rhea* clearly indicates the presence of a gonial similarly situated in relation to the articular cartilage and the angular and surangular as in *Struthio*. Meckel's cartilage shows no signs of ossification anteriorly where it is indicated by a very thin rod; posteriorly ossification of the articular cartilage has not commenced.

Struthio, Crypturellus and *Rhea* together with *Dromiceius* (PARKER, 1866) and *Apteryx* (PARKER, 1891), all have five dermal bones in the lower jaw. The median proximal one is the gonial. Meckel's cartilage only ossifies as the articular from the pars articularis; the rest is absorbed.

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EYE MUSCLES, INNERVATION AND BLOOD SUPPLY

There are no interesting deviations from the normal relations of the ocular muscles typical of vertebrates.

The nictitating muscles, of which there are two, the m.quadratus and m.pyramidalis, are important in operating the membrana nictitans. Both these muscles are innervated by the n.abducens (Fig. 42). They are closely applied to the surface of the orbit, the m.quadratus being the larger and more caudally situated, and lying dorsal to the n.opticus. In shape the m.quadratus is triangular, with the apex of the triangle lying immediately dorsal to the n.opticus (Fig. 42). The apex is in the form of a loop through which passes the tendon of the m. pyramidalis (Fig. 43). The loop of the m.quadratus and the tendon of the m.pyramidalis develop sometime after these two muscles have been laid down and so appear relatively late in ontogeny. The ventral margin of the loop be-

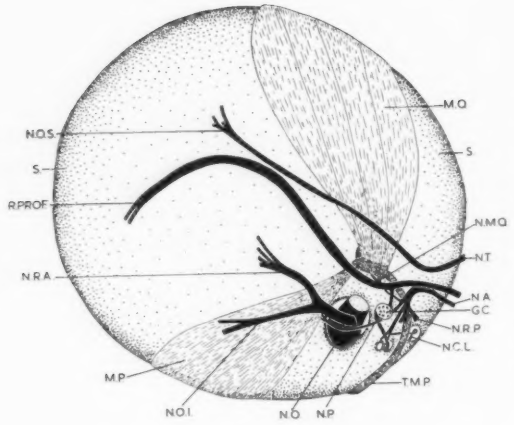

Fig. 42. Graphic reconstruction illustrating the nerves and nictitating muscles of the eye, in the 27-day old embryo. × 5. Medial aspect. *G.C.*, g.ciliare; *M.P.*, m. pyramidalis; *M.Q.*, m.quadratus; *N.A.*, n.abducens; *N.C.L.*, n.ciliaris longi; *N.M.Q.*, n.m.quadratus; *N.O.*, n.opticus; *N.O.I.*, n.obliquus inferior; *N.O.S.*, n.obliquus superior; *N.P.*, n.pyramidalis; *N.R.A.*, n.rectus anterior; *N.R.P.*, n.rectus posterior; *N.T.*, n.trochlearis; *R.PROF.*, r.profundus; *S.*, sclera; *T.M.P.*, tendon of m.pyramidalis.

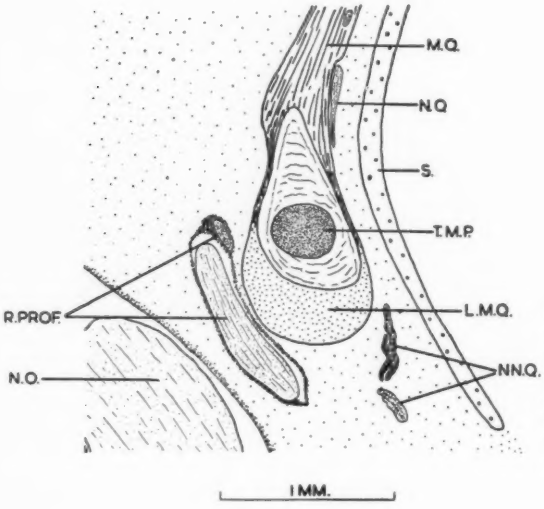

Fig. 43. Cross-section through the loop of the m.quadratus in the 37-day old embryo. *L.M.Q.*, loop of m.quadratus; *M.Q.*, m. quadratus; *N.N.Q.*, n.quadratus; *N.O.*, n.opticus; *N.Q.*, n.quadratus; *R.PROF.*, r.profundus; *S.*, sclera; *T.M.P.*, tendon of m. pyramidalis.

comes considerably thickened, until in the 37-day old embryo it has become chondrified (Fig. 43).

The tendon of the m.pyramidalis, after passing through the loop of the m.quadratus, follows a course ventrally and orally and finds attachment on the postero-ventral corner of the membrana nictitans (Figs. 42 and 44). STRESEMANN (1927—1934) has given a brief but accurate description of the structure and mechanism of the membrana nictitans, and his findings also seem to apply to *Struthio*.

The innervation of the m.quadratus and m.pyramidalis is by a branch of the n.abducens which becomes very clearly divided into a ramus ventralis innervating the posterior rectus, and a ramus dorsalis giving off two branches

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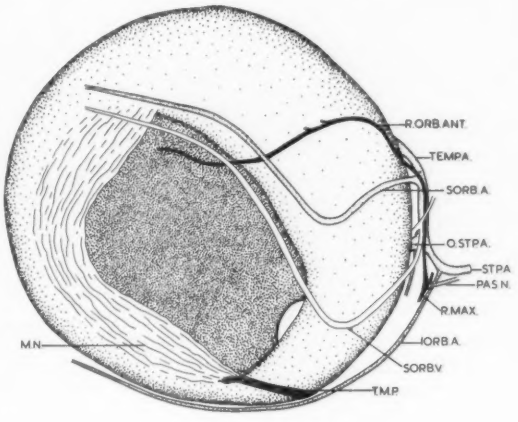

Fig. 44. Graphic reconstruction of the scleral cartilage illustrating the nerves and blood vessels in the 27-day old embryo. × 5. Lateral aspect. *IORB.A.*, infraorbital artery; *M.N.*, membrana nictitans; *O.STP.A.*, ophthalmic branch of stapedial artery; *PAS.N.*, parasympathetic nerve; *R.MAX.*, r.maxillaris; *R.ORB.ANT.*, r.orbitalis anterior; *SORB.A.*, supraorbital artery; *SORB.V.*, supraorbital vein; *STP.A.*, stapedial artery; *TEMP.A.*, temporal artery; *T.M.P.*, tendon of m.pyramidalis.

supplying nerves to the muscles operating the membrana nictitans. The nerve to the m.pyramidalis follows a direct course passing close to the g.ciliare and then orally medial to the optic nerve (Fig. 42). The branch innervating the m.quadratus is short and passes dorsally, lateral to the r.profundus Va and enters the muscle laterally, (Figs. 42 and 43). In its course it becomes divided into a large number of branches which are enclosed in dense connective tissue; before entering the muscle these unite and form a single nerve (Fig. 42). The reason for the n.quadratus being broken up into a large number of radicles, which again unite, is not clear. This feature has not been previously described.

In contradistinction to the m.depressor palpebrae inferioris which operates the lower eyelid, there is a muscle on the dorsal side of the bulbus which effects the lifting of the upper eyelid and is known as the m.levator palpebrae superioris. It is attached postero-dorsally to the orbit and extends into the hinder part of the upper lid.

The exact innervation of this muscle could not be determined, but is it quite certain that the innervation is not from any of the three somatic motor nerves innervating the eye muscles. It is possible that the innervation is from the n.trigeminus: the n.maxillaris (Vb) in its course through the rete mirabile ophthalmicum (rete temporale—HUGHES, 1934) gives off a branch which passes dorsally through the rete to become the r.supraorbitalis and then passes lateral to the eye and under the m.levator palpebrae superioris in its course rostrally. At a point where the r.supraorbitalis leaves the r.maxillaris, it is joined by a parasympathetic nerve (Fig. 44), but the extent to which the r.supraorbitalis possesses parasympathetic fibres in its course is exceedingly difficult to determine. The r.supraorbitalis, however, gives off a number of radicles in the vicinity of the m.levator palpebrae superioris, but it cannot be established whether these radicles enter the muscle. They probably come from the para-

sympathetic nervous system, as the r.maxillaris itself contains only somatic sensory elements.

In birds nothing has been done in this connexion. However, DE VILLIERS (1938, p. 17) states for *Scolecomorphus* that there "is a short but very thick ramus of Vb which seems to innervate the m.adductor mandibulae externus near its cranial insertion." On p. 19 he states for *Boulengerula:* "... Vb sends a short thick ridge-like branch, which goes to the musculus adductor mandibulae externus at its dorso-medial insertion.", and further, on the same page: "... of *Boulengerula,* Vb is again a sensory motor nerve supplying the m.adductor mandibulae externus and the ventral skin of the snout." In these *Gymnophiona,* therefore, Vb possesses motor fibres which innervate a muscle of the trigeminus group: the m.adductor mandibulae externus. In *Struthio,* the same muscle, though divided into two definite portions, is innervated from Vc.

It seems quite likely that the branch of Vb innervating the m.adductor mandibulae externus in the *Gymnophiona,* has received motor fibres from Vc. In *Struthio* the condition is different for a number of reasons: firstly, Vb has a supraorbital branch which goes to the skin above the orbit, and which therefore contains somatic sensory elements, while in the *Gymnophiona* the branch of Vb seems to go to the muscle only. Secondly, since there are a number of radicles underlying the m.levator palpebrae superioris which are branches of the r.supraorbitalis, and since this nerve is joined by a parasympathetic nerve at its origin with Vb, it seems possible that the radicles are parasympathetic and not branches of the r.supraorbitalis.

NISHI (1938) is of the opinion that the m.levator palpebrae superioris is a derivative of the rectus superior, in which case one would expect it to be innervated by the oculomotor. But the innervation of this muscle is not from the somatic motor nerves of the eye muscles. It must therefore get its innervation from the r.supraorbitalis which contains motor elements (cf. DE VILLIERS, 1938), or from the parasympathetic nervous system. This is more likely, since the r.maxillaris and r.supraorbitalis both pass through the rete mirable ophthalmicum and are joined by parasympathetic nerves.

BLOOD SUPPLY TO THE EYE AND EYE MUSCLES

In *Struthio* there is no discreet retinal artery; the retina receives its blood from the a.ophthalmica externa which sends off a number of small branches which pass through their own foramina in the cartilaginous orbit. LILLIE (1919) was the first to notice that in birds there is no central retinal artery, and HUGHES (1934) in his work on the chick, states that arterial blood for the eye is supplied through a number of small arteries which are branches of the ophthalmic trunk.

All the eye muscles except the m.levator palpebrae superioris are supplied

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with blood from the a.ophthalmica externa, which branches off from the a.stapedia. The a.ophthalmica externa follows a rostral course, passing first of all slightly ventrally so as to lie medial to the n.opticus. It gives off a small single branch to the rectus temporalis, (rectus posterior) and then a larger branch to the m.rectus superior and m.obliquus superior. Anterior to the n. opticus a single branch goes to the m.rectus inferior with the artery ending in three main ramuli: a dorsal branch going to the rectus nasalis (m.rectus anterior), a ventral one going to the m.obliquus inferior, and the medial branch going to the Harderian gland.

Blood to the m.levator palpebrae superioris is supplied through ramuli of the supraorbital artery, a branch of the stapedial artery.

TRIGEMINUS MUSCULATURE

A. CONSTRICTOR I DORSALIS

(1) M. Protractor Quadrati

This is a single muscle, well-developed and situated between the basisphenoid and processus orbitoquadratus. It arises, for the greater part, on the lateral surface of the basisphenoid to which it is broadly attached. The muscle is quite extensive in the horizontal plane and extends between the foramen opticum anteriorly and foramen prooticum posteriorly. The dorsal margin of the muscle is in line with the dorsal limit of the basisphenoid, and ventrally it extends practically to its latero-ventral margin (Figs. 45 and 46). Postero-dorsally the muscle arises not only from the basisphenoid, but also from the ventral margin of the pleurosphenoid. It thus covers the foramen prooticum and is penetrated by Vb + Vc. The greater portion of this muscle is, however, mediad to Vb.

The fibres of the m.protractor quadrati pass latero-ventrally and slightly caudally, becoming inserted on the median surface of the processus orbito-quadratus. The antero-rostral fibres are inserted on the medio-dorsal surface of the processus orbitquadratus, while the ventrocaudal fibres are inserted on the corpus ossis-quadrati (Figs. 45 and 46).

(2) M. Levator Bulbi

According to LAKJER (1926) this muscle is homologous with the pars dorsalis of the m.protractor quadrati of reptiles, and is represented by two very distinct muscle plates which are distinguished by their insertions and the direction of the fibres. LAKJER (1926) referred to the dorsal plate as the m.tensor periorbitae, and the ventral the m.depressor palpebrae inferioris.

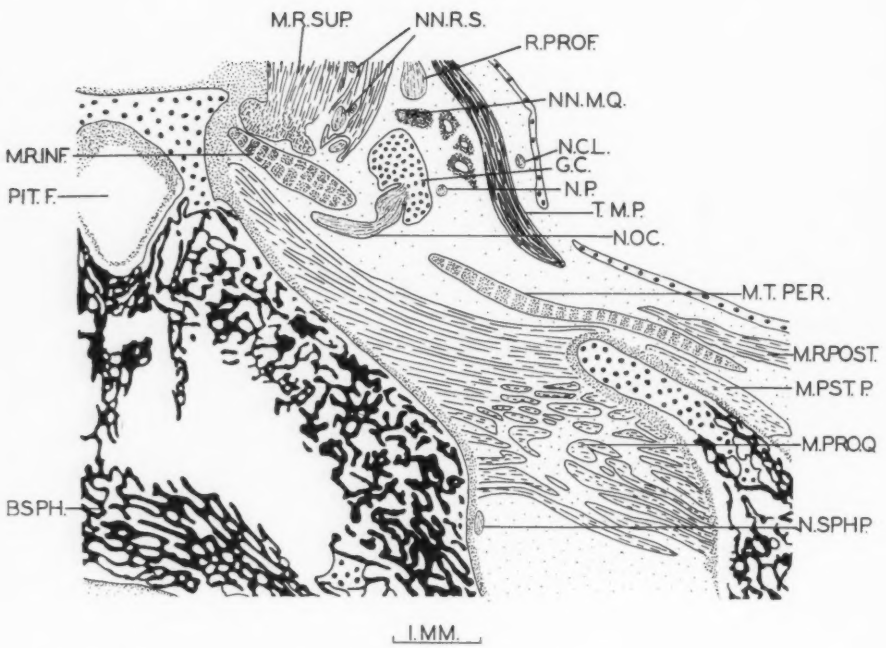

Fig. 45. Transverse section through the basisphenoid and the quadrate in the region of the g.ciliare, in the 37-day old embryo. *BSPH.*, basisphenoid; *G.C.*, g.ciliare; *M.PRO.Q.*, m.protractor quadrati; *M.PST.P.*, m.pseudotemporalis profundus; *M.R.INF.*, m.rectus inferior; *M.R.POST.*, m.rectus posterior; *M.R.SUP.*, m.rectus superior; *M.T.PER.*, m.tensor periorbitae; *N.C.L.*, n.ciliaris longi; *N.N.M.Q.*, nerves to m.quadratus; *N.N.R.S.*, nerves to rectus superior; *N.OC.*, n.oculomotorius; *N.P.*, n.pyramidalis; *N.SPHP.*, n.sphenopalatinus; *PIT.F.*, pituitary fossa; *R.PROF.*, r.profundus; *T.M.P.*, tendon of the m.pyramidalis.

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(a) *M.Tensor Periorbitae*

This muscle lies medial to the r.maxillaris and its fibres pass rostrally in the horizontal plane. It arises from the antero-lateral surface of the pleurosphenoid immediately dorsal to the pleurosphenoid-basisphenoid suture, and immediately above the m.protractor quadrati. It then extends from a point immediately anterior to the foramen prooticum to one immediately posterior to the lateral opening of the foramen profundum. The fibres become inserted on an aponeurosis ventral to the bulbus oculi, and pass at right angles to those of the m.dep.pal. inferioris. Posteriorly the muscle is dorsal to the m.protractor quadrati and ventral to the m.rectus posterior (Fig. 46). Anteriorly it lies between the m.dep.pal. inferioris below, and the m.rectus inferior above (Fig. 47). In *Spheniscus demersus* (Prins, 1951) this muscle occupies an identical position, arising and inserting in the same manner as described above for *Struthio*.

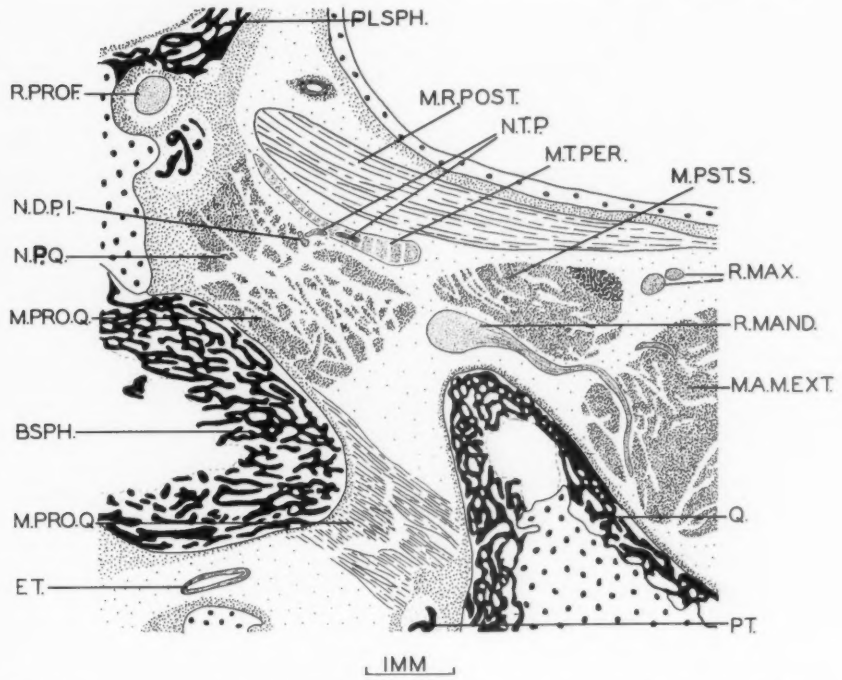

Fig. 46. Transverse section through the basisphenoid and quadrate in the 37-day old embryo. *BSPH.*, basisphenoid; *E.T.*, eustachian tube; *M.A.M.EXT.*, m.adductor mandibulae externus; *M.PRO.Q.*, m.protractor quadrati; *M.PST.S.*, m.pseudotemporalis superficialis; *M.R.POST.*, m.rectus posterior; *M.T.PER.*, m.tensor periorbitae; *N.D.P.I.*, n.depressor palpebrae inferioris; *N.P.Q.*, n.protractor quadrati; *N.T.P.*, n.tensor periorbitae; *PLSPH.*, pleurosphenoid; *PT.*, pterygoid; *Q.*, quadrate; *R.MAND.*, r.mandibularis; *R.MAX.*, r.maxillaris; *R.PROF.*, r.profundus.

(b) *M.Dep.Pal. Inferioris (Fig. 47)*

This muscle arises on the interorbital septum and extends from a point anterior to the foramen opticum to the anterior limit of the m.protractor quadrati. It is broadly attached to the septum immediately dorsal to the m.protractor quadrati, and the fibres pass laterally to the lower eyelid where they find their insertion. The muscle is typically triangular in shape, the apex being the point of attachment to the skull. In *Spheniscus* (Prins, 1951) there is a similar arrangement of this muscle.

Innervation

The derivatives of the constrictor I dorsalis are supplied from a single branch of Vc which arises ventrally from Vc before Vb joins it to form the truncus maxillomandibularis (Fig. 48 A and B). The n.constrictor I dorsalis

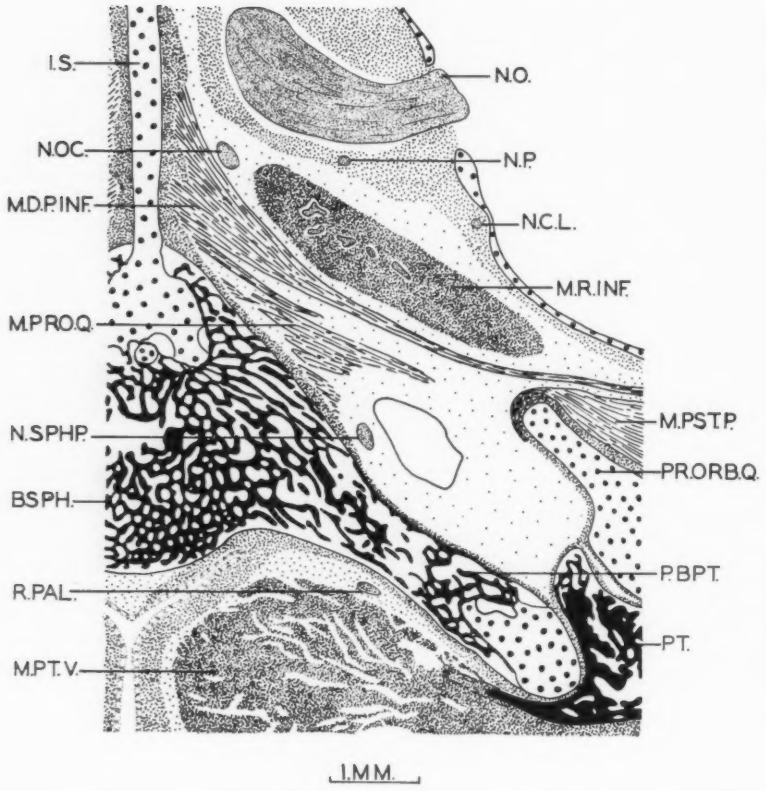

Fig. 47. Transverse section through the processus basipterygoideus in the 37-day old embryo. *BSPH.*, basisphenoid; *I.S.*, interorbital septum; *M.D.P.INF.*, m.depressor palpebrae inferioris; *M.PRO.Q.*, m.protractor quadrati; *M.PST.P.*, m. pseudotemporalis profundus; *M.PT.V.*, m.pterygoideus ventralis; *M.R.INF.*, m.rectus inferior; *N.C.L.*, n.ciliaris longi; *N.O.*, n.opticus; *N.OC.*, n.oculomotorius; *N.P.*, n.pyramidalis; *N.SPHP.*, n.sphenopalatinus; *P.BPT.*, processus basipterygoideus; *PR.ORB.Q.*, processus orbitoquadratus; *PT.*, pterygoid; *R.PAL.*, r.palatinus.

divides into two main branches: the ventral branch, n.protractor quadrati, supplies the muscle of the same name, and the dorsal branch, n.levator bulbi, passes dorsally through the m.protractor quadrati where it divides into the n.tensor periorbitae and n.dep.pal. inferioris (Fig. 48 A and B). The n.dep.pal. inferioris passes rostrally ventral to the m.tensor periorbitae to enter the m.dep.pal. inferioris at its point of origin.

In *Spheniscus* the n.constrictor I dorsalis arises directly from the g.gasseri on the medio-ventral surface (Prins, 1951), while in *Struthio* this nerve branches off from Vc.

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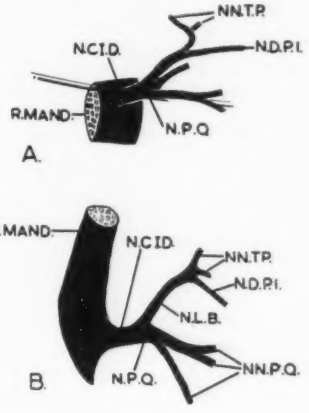

Fig. 48. Contour reconstructions of the branches of the n.constrictor I. dorsalis in the 27-day old embryo. × 15. A) Medial aspect. B) Ventral aspect. *N.C.I.D.*, n.constrictor I dorsalis; *N.D.P.I.*, n.depressor palpebrae inferioris; *N.L.B.*, n.levator bulbi; *N.N.P.Q.*, nervi protractor quadrati; *N.N.T.P.*, nervi tensor periorbitae; *N.P.Q.*, n.protractor quadrati; *R.MAND.*, r.mandibularis.

B. MUSCULUS ADDUCTOR MANDIBULAE

There are four muscles belonging to this division; their grouping is based upon the position of the muscles relative to the r.maxillaris and r.mandibularis. This is the substance of LUTHER's (1914) classification, which divides the m.adductor mandibulae into three topographical parts termed by him (1914): m.adductor posterior, m.adductor internus and m.adductor externus. SÄVE—SÖDERBERGH (1944) has described the positions relative to the r.mandibularis and r.maxillaris as follows: the posterior position, posterior or medial to the r.mandibularis (and r.maxillaris), the externus position, lateral to the r.mandibularis and the r.maxillaris, and the internus position anterior or medial to the r.maxillaris but lateral to the r.profundus.

In *Struthio* the four muscles of the adductor division can be classified depending on their position relative to the r.maxillaris and r.profundus. Three of the muscles belong to the internus division inasmuch as they lie medial to the r.maxillaris and lateral to the r.profundus. The remaining muscle belongs to the external division because its position is lateral to both the r.maxillaris and the r.mandibularis. In *Struthio,* therefore, there is present a m.adductor mandibulae externus group, and a m.adductor mandibulae internus group, the posterior group being absent.

(1) M. Adductor Mandibulae Externus

This muscle lies wholly lateral to the r.mandibularis, it is moderately large and arises on the zygomatic process of the squamosal (Fig. 49). The fibres pass in an antero-ventral direction to become inserted onto the lateral surface of the surangular, in the course of which it passes medial to the lower temporal arch in the region of the jugal-quadratojugal arthrosis (Fig. 49).

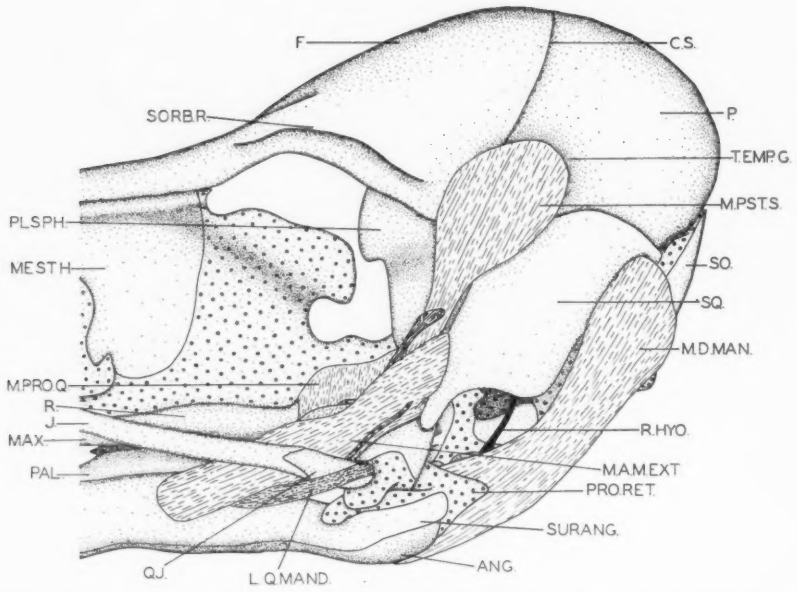

Fig. 49. Graphic reconstruction of the posterior part of the skull in the 37-day old embryo to show the trigeminus and facial musculature. × 3·3. Laterial aspect. *ANG.*, angular; *C.S.*, coronal suture; *F.*, frontal; *J.*, jugal; *L.Q.MAND.*, ligamentum quadrato-mandibularis; *M.A.M.EXT.*, m.adductor mandibulae externus; *MAX.*, maxillary; *M.D.MAN.*, m.depressor mandibulae; *MESTH.*, mesethmoid; *M.PRO.Q.*, m.protractor quadrati; *M.PST.S.*, m.pseudotemporalis superficialis; *P.*, parietal; *PAL.*, palatine; *PLSPH.*, pleurosphenoid; *PRO.RET.*, processus retroarticularis; *QJ.*, quadratojugal; *R.*, rostrum; *R.HYO.*, r.hyomandibularis; *SO.*, supra-occipital; *SORB.R.*, supraorbital ridge; *SQ.*, squamosal; *SURANG*, surangular; *TEMP.G.*, temporal groove.

The muscle is peculiar because approximately in the middle of its postero-ventral surface there is a well-developed aponeurosis. On the dorsal surface there is another aponeurosis which extends from the point of insertion to one diametrically opposite the ventral beginning of the postero-ventral aponeurosis. The dorsal aponeurosis is superficial, while the ventral one penetrates some distance into the substance of the muscle. The posterior part of the muscle therefore, when viewed in cross-section, shows three distinct regions, separated by a T-shaped aponeurosis (Fig. 50). This division of the muscle is of no morphological significance as the three regions are in histological continuity, and do not represent fused sub-divisions of the m.adductor mandibulae group.

Innervation

The innervation of the m.adductor mandibulae externus is from a large number of radicales derived mainly form a single branch of Vc before the

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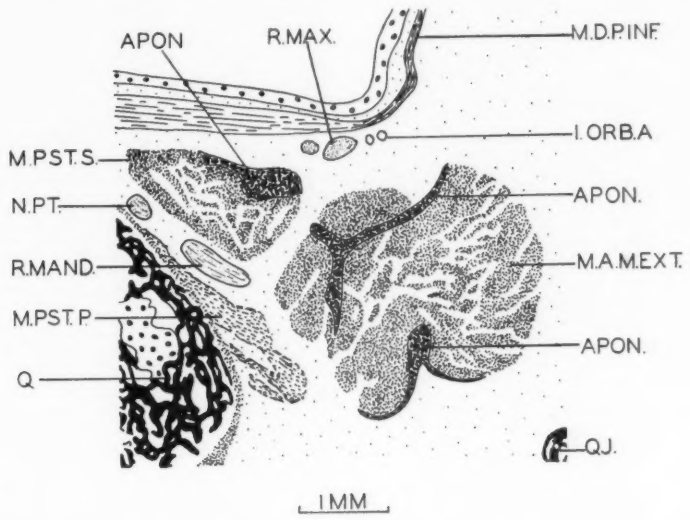

Fig. 50. Transverse section through the m.adductor mandibulae externus in the 37-day old embryo. *APON.*, aponeurosis; *IORB.A.*, infraorbital artery; *M.A.M.EXT.*, m.adductor mandibulae externus; *M.D.P.INF.*, m.depressor palpebrae inferioris; *M.PST.P.*, m.pseudotemporalis profundus; *M.PST.S.*, m.pseudotemporalis superficialis; *N.PT.*, n.pterygoideus; *Q.*, quadrate; *Q.J.*, quadratojugal; *R.MAND.*, r.mandibularis; *R.MAX.*, r.maxillaris.

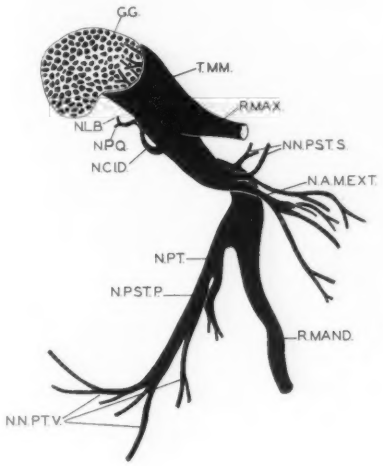

Fig. 51. Contour reconstruction of the motor nerves of V of the 27-day old embryo. × 11·1. Posterior aspect. *G.G.*, g.gasseri; *N.C.I.D.*, n.constrictor I dorsalis; *N.L.B.*, n.levator bulbi; *N.N.PST.S.*, nerves to pseudotemporalis superficialis; *N.N.PT.V.*, nerves to pterygoideus ventralis; *N.P.Q.*, n.protractor quadrati; *N.PT.*, n.pterygoideus; *N.PST.P.*, n.pseudotemporalis profundus; *R.MAND.*, r.mandibularis; *R.MAX.*, r.maxillaris; *T.MM.*, truncus maxillo-mandibularis.

n.pterygoideus is given off. Apart from these radicles, the muscle also receives a radicle from a branch of Vc going to the m.adductor mandibulae internus (Fig. 51). All the nerves entering the muscle are medially placed and enter the muscle more or less half way between origin and insertion (Fig. 46).

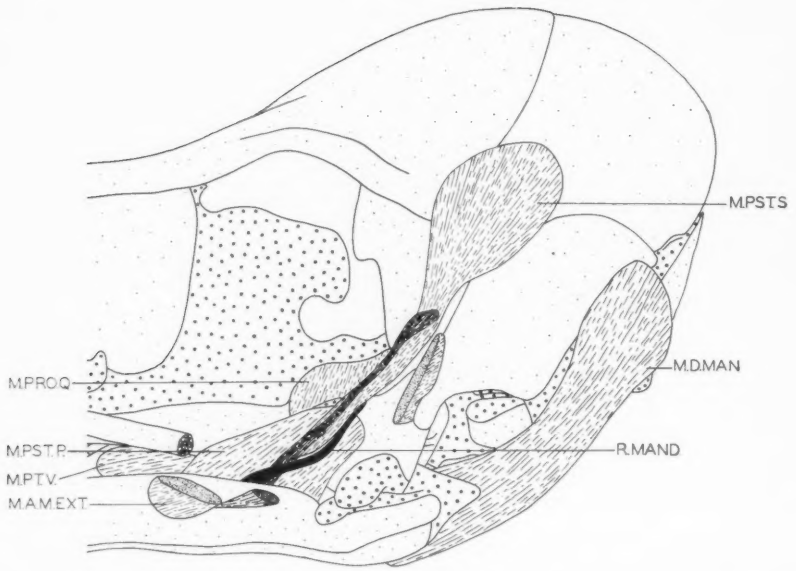

Fig. 52. Graphic reconstruction of the posterior part of the skull of the 37-day old embryo. (The m.adductor mandibulae externus removed). × 3·3. Lateral aspect. *M.A.M.EXT.*, m. adductor mandibulae externus; *M.D.MAN.*, m.depressor mandibulae; *M.PRO.Q.*, m.protractor quadrati; *M.PST.P.*, m.pseudotemporalis profundus; *M.PST.S.*, m.pseudotemporalis superficialis; *M.PT.V.*, m.pterygoideus ventralis; *R.MAND.*, r.mandibularis.

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(2) M. Adductor Mandibulae Internus

There are three sections of the adductor group, all of which are medial to the r.maxillaris and lateral to the r.profundus. The three sections present in *Struthio* are: m.pseudotemporalis superficialis; m. pseudotemporalis profundus and m.pterygoideus.

(i) *M.Pseudotemporalis Superficialis*

This is the second largest of the adductor mandibulae muscles. Dorsally it is broad and fan-shaped, and is typically temporal in position, having its origin on the temporal groove, thus covering sections of the frontal, parietal and squamosal. It is also attached to the posterior part of the pleurosphenoid, this attachment beginning immediately dorsal to the anterior end of the foramen prooticum. From the point of origin of the muscle the fibres pass ventrally and only slightly anteriorly. (Fig. 52).

The ventral part of the muscle is, for the greater part, triangular in cross-section (Figs. 46 and 50), with the latero-dorsal margin of the muscle covered by an extensive aponeurosis which is continued ventrally to form a strong tendon by means of which the muscle becomes inserted

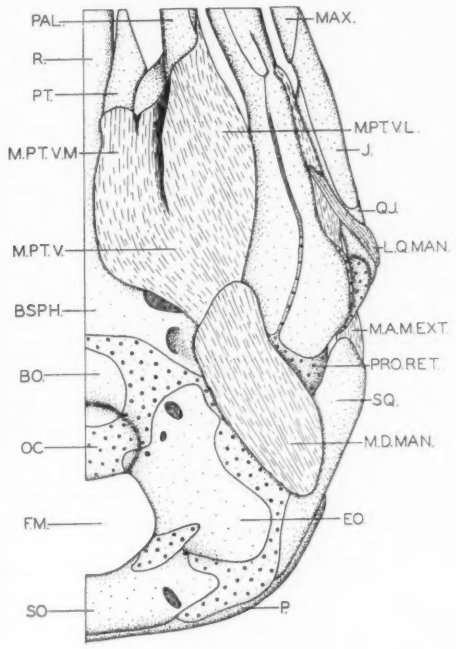

Fig. 53. Graphic reconstruction of the posterior part of the skull of the 37-day old embryo. × 3·3. Ventral aspect. *BO.*, basioccipital; *BSPH.*, basisphenoid; *EO.*, exoccipital; *F.M.*, foramen magnum; *J.*, jugal; *L.Q.MAN.*, ligamentum quadrato mandibularis; *M.A.M.EXT.*, m.adductor mandibulae externus; *MAX.*, maxillary; *M.D.MAN.*, m.depressor mandibulae; *M.PT.V.*, m.pterygoideus ventralis; *M.PT.V.L.*, m.pterygoideus ventralis lateralis; *M.PT.V.M.*, m.pterygoideus ventralis medialis; *O.C.*, occipital condyle; *P.*, parietal; *PAL.*, palatine; *PRO.RET.*, processus retroarticularis; *PT.*, pterygoid; *QJ.*, quadratojugal; *R.*, rostrum; *SO.*, supraoccipital; *SQ.*, squamosal.

onto the medial surface of the surangular. A portion of the m.pseudotemporalis is attached to this tendon.

Innervation of the M.Pseudotemporalis Superficialis

The m.adductor mandibulae internus is innevated by branches of Vc which leave Vc just before it is joined by Vb (r.maxillaris). Apparently only two branches innervate this muscle, and both enter it ventrally near its attachment to the pleurosphenoid. One of the branches that innervates the m.adductor mandibulae internus arises together with a branch that innervates the m.adductor mandibulae externus (Fig. 51).

(ii) *M.Pseudotemporalis Profundus (Fig. 52)*

The fibres of this muscle pass in a ventro-lateral direction extending from their origin on the antero-lateral surface of the processus orbitoquadratus, to their insertion on the dorso-medial surface of the mandible, finding attachment partially on the articular and for a considerable distance on the surangular as well as on the dorsal surface of the angular. The insertion is extensive, being about the same length as the pterygoid.

(iii) *M.Pterygoideus (Fig. 53)*

In birds this muscle generally consists of two components, the m.pterygoideus dorsalis and the m.pterygoideus ventralis. In *Struthio* only the

ventralis component is present but, despite this lack of the m.pterygoideus dorsalis, the m.pterygoideus remains the largest and most strongly developed muscle of the whole of the adductor mandibulae group.

(3) M. Pterygoideus Ventralis

For the greater part, this muscle is ventrally placed and the fibres pass caudally and slightly laterally; anteriorly there are two very distinct sections, one medial and one lateral, they will be referred to as the m.pterygoideus ventralis medialis and m.pterygoideus ventralis lateralis.

The antero-lateral portion of the m.pterygoideus ventralis lateralis arises on the dorso-lateral and ventro-lateral surfaces of the palatines; and the fibres pass in a ventrolateral and slightly caudal direction to become inserted onto the dorso-medial surface of the mandible (medial to the m.pseudotemporalis profundus) on the angular and on the gonial. The anterior portion of the m.pterygoideus ventralis medialis arises on the medio-ventral surface of the palatine and on the ventral surface of the pterygoid. The fibres of this portion of the muscle pass directly caudally. These two sections of the m.pterygoideus ventralis remain separate for about half the extent of the muscle owing to the presence of an aponeurosis. The medial part of the m.pterygoideus ventralis lateralis overlies the lateral margin of the m.pterygodieus ventralis medialis (Fig. 54).

In the posterior half of the muscle the components have become united into a single entity, with the fibres passing in the same direction: ventro-laterally and slightly caudally. The insertion of the muscle is extensive, being medial to the m.pseudotemporalis profundus, and inserting for the greater part on the gonial anteriorly and the angular posteriorly. The extreme posterior end of the muscle is peculiar inasmuch as in both embryo and adult, the muscle becomes divided into three very distinct portions: dorsal, medial and ventral. The ventral portion is terminally inserted on the angular, while the medial and dorsal sections are inserted on, as yet, unossified articular cartilage—the processus mandibularis internus (Fig. 55), in the embryo. In some of the adults, it was found that the dorsal portion is not terminally inserted on the mandible, but becomes attached by means of a long tendon to the processus paroccipitalis of the exoccipital.

Innervation of the M.Pseudotemporalis Profundus and M.Pterygoideus.

The innervation of the m.adductor mandibulae internus group is from the n.pterygoideus of the r.mandibularis. Fig 51 illustrates the branches of Vc from which arise the branches that innervate the trigeminus musculature. Fundamentally the main branches remain the same in all the embryos studied, with only the smaller branches showing variation. The n.pterygoideus is a large

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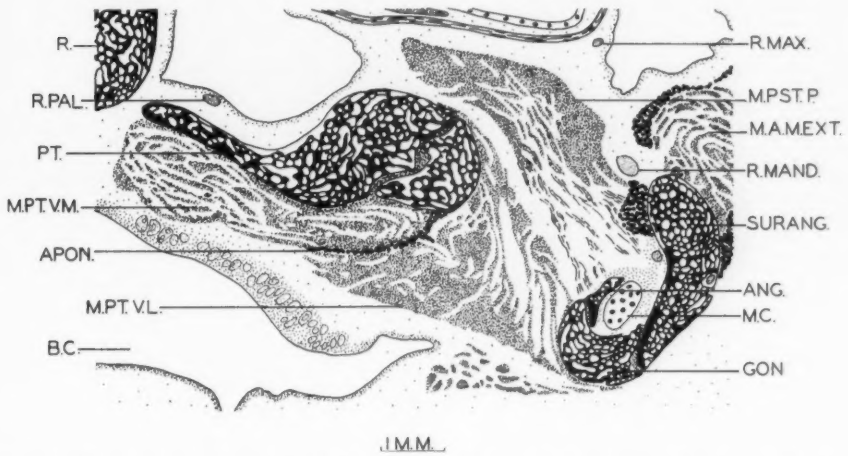

Fig. 54. Transverse section through the region of the palatine-pterygoid arthrosis in the 37-day old embryo. *ANG.*, angular; *APON.*, aponeurosis; *B.C.*, buccal cavity; *GON.*, gonial; *M.A.M.EXT.*, m.adductor mandibulae externus; *M.C.*, Meckel's cartilage; *M.PST.P.*, m.pseudotemporalis profundus; *M.PT.V.L.*, m.pterygoideus ventralis lateralis; *M.PT.V.M.*, m.pterygoideus ventralis medialis; *PT.*, pterygoid; *R.*, rostrum; *R.MAND.*, r.mandibularis; *R.MAX.*, r.maxillaris; *R.PAL.*, r.palatinus; *SURANG.*, surangular.

branch of Vc, and it passes for a short distance anteriorly, lateral to the posterior part of the m.pseudotemporalis profundus. It then enters the muscle and gives off a small, short branch—the n.pseudotemporalis profundus (Fig. 51) innervating the muscle of the same name. The bulk of the fibres constituting the n.pterygoideus pass ventro-medially through the m.pseudotemporalis profundus, and on reaching the margin of the m.pterygoideus ventralis, break up into a number of radicles which innervate the muscle.

Discussion

The cranial musculature, more particularly the trigeminus musculature, has been studied by such workers as EDGEWORTH (1907) (development of the cranial muscles in *Gallus domesticus*), and LAKJER (1926) whose work on the trigeminus musculature of *Sauropsida* can be regarded as the standard work on this group of muscles for birds. Since that time workers such as MOLLER (1930), PRINS (1951), FIEDLER (1951) and BARNIKOL (1953 and 1953 a) have added considerably to our knowledge of the trigeminus musculature of birds.

In comparing the trigeminus musculature of *Struthio* with that of the birds previously studied, it is clear that *Struthio* possesses a very much simplified adductor mandibulae complex, and has only four separate muscles, whereas in the *Neognathae*, so far studied, the m.adductor mandibulae complex possesses a far greater number of separate divisions.

The condition in *Struthio* can be regarded as being either a specialization to feeding habits, or as being neotenic.

An explanation of the condition in *Struthio* will be based on previous works on mainly adult avian material. For the ontogeny reference can only be made to EDGEWORTH (1907) and PRINS (1951).

Early in the ontogeny of *Spheniscus* (61 mm.) a stage in the development of the trigeminus musculature is reached which resembles that of the late embryo and adult of *Struthio*. In this stage of *Spheniscus* the muscle anlage has become differentiated and is in definite relation to the skeletal elements. The muscles retain their primary position in regard to their origin and insertion (PRINS, 1951). Moreover, the m.adductor mandibulae externus and internus divisions are clearly separated, with the internus division being divided into the pseudotemporalis and pterygoideus groups. The latter group is divided into dorsal and ventral components which are, however, not clearly separated from each other, but the course followed by the fibres differs considerably (PRINS, 1951).

Struthio, though possessing the pterygoideus group of the internus division of the adductor mandibulae, yet lacks the dorsal component. The ventral component of the m.pterygoideus in the 61 mm. stage of *Spheniscus* is divided anteriorly into a m.pterygoideus ventralis lateralis and a m.pterygoideus ventralis medialis (PRINS, 1951). This subdivision of the m.pterygoideus ventralis is present in the late embryo and adult of *Struthio*. In the adult of *Spheniscus*, the m.pterygoideus ventralis medialis is divided into dorsal and ventral sections (PRINS, 1951). This is also the case in *Struthio* but is confined to the extreme posterior end of the muscle, at the point of insertion (Fig. 55).

The pseudotemporalis group of the internus division of the m.adductor mandibulae in *Struthio* and *Spheniscus* is divided into superficialis and profundus sections.

The m.adductor mandibulae externus in the 61 mm. stage of *Spheniscus* is undifferentiated into components (PRINS, 1951) which is the condition in late embryos and in the adult of *Struthio*. In the adult of *Spheniscus* the m.adductor mandibulae externus is divided into there very distinct muscle masses, forming the superficialis, medialis and profundus sections of the externus division. This sub-division of the externus division is also present in *Sericotes*, *Cinnyris* and *Anthornis* (MOLLER, 1930). FIEDLER (1951) found that the externus division is also subdivided in the *Oscines*, but he referred to the sub-division as rostral, caudal and ventral. The nomenclature adopted is not important, but what is important is that whereas the m.adductor mandibulae externus is divided into three very definite sections in all these neognathic birds, it is single in *Struthio*, so that the pattern appears neotenic.

The externus division of the m.adductor mandibulae is sub-divided not only in neognathic birds, but also in *Lacertilia*, *Rhynchocephalia*, *Ophidia*, *Crocodilia* and *Chelonia* where it is differentiated into the superficialis, medialis and pro-

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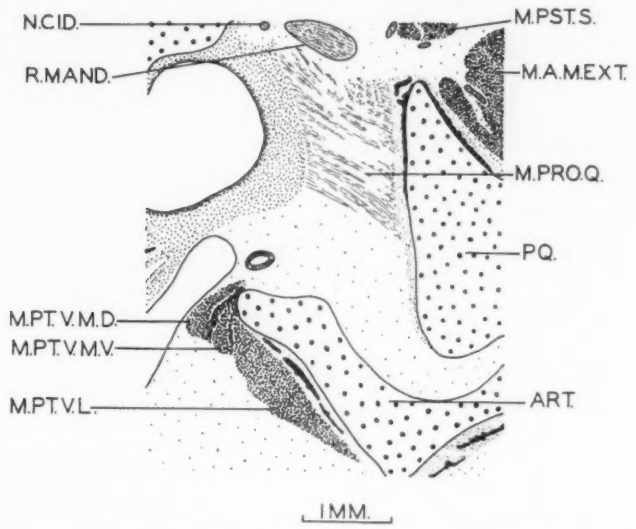

Fig. 55. Transverse section through the posterior part of the articular cartilage in the 27-day old embryo. *ART.*, articular; *M.A.M.EXT.*, m.adductor mandibulae externus; *M.PRO.Q.*, m.protractor quadrati; *M.PST.S.*, m.pseudotemporalis superficialis; *M.PT.V.L.*, m.pterygoideus ventralis lateralis; *M.PT.V.M.D.*, m.pterygoideus ventralis medialis dorsalis; *M.PT.V.M.V.*, m.pterygoideus ventralis medialis ventralis; *N.CI.D.*, n.constrictor I dorsalis; *P.Q.*, pars quadrata; *R.MAND.*, r.mandibularis.

fundus layers (LAKJER, 1926). In *Anura* too, it is large and differentiated into three layers (SÄVE—SÖDERBERGH, 1944). The undivided condition of the muscle in *Struthio* can therefore hardly be termed primitive, but must either be regarded as specialized or neotenic. If the condition is a specialization, it must be associated with the feeding habits of the bird, which are of a grazing nature, thus eliminating the necessity for well-developed mandibular adductors. This would then be a "specialization through reduction", especially as the m.adductor mandibulae externus shows along its length an aponeurosis that incompletely divides the muscle into three divisions (Fig. 50) which might represent the superficialis, medialis and profundus sections of *Anura, Reptilia* and the *Neognathae*.

It is, however, quite conceivable that the condition in *Struthio* of a single m.adductor mandibulae externus, illustrates the phenomenon of neoteny. This contention is based on two arguments, one being that *Spheniscus,* during ontogeny, illustrates the singleness of the m.adductor mandibulae externus. The second is that *Struthio* has been shown to be neotenic on other anatomical features, so that partial neoteny affecting the muscle pattern may reasonably be expected.

The same argument may also be applied to the m.pterygoideus ventralis component of the m. adductor mandibulae internus division, because in *Struthio*

the m.pterygoideus ventralis is only partially divided into lateral and medial sections with the latter further partially divided into dorsal, medial and ventral sections, while in *Spheniscus* these divisions are complete.

In contrast to the phenomenon of neoteny, the idea that *Struthio* illustrates advanced specialization through reduction, because of the feeding habit, is supported to some extent by the absence of some of the adductor mandibulae elements. The muscles not represented are the m.adductor mandibulae posterior and m.pterygoideus dorsalis. Whether these two muscles have been lost or whether they have been incorporated into, or not separated off by aponeuroses from, the surrounding muscles is very difficult to determine. At no time during the ontogeny of the trigeminus musculature of *Struthio* are there more muscles than there are in the 37-day old embryo, or in the adult.

C. CONSTRICTOR I VENTRALIS

M.Intermandibularis

This muscle is in the form of a thin plate having its origin posteriorly on the dorso-medial surface of the surangular and anteriorly on the medial surface of the postero-dorso-medial process of the dentary. The fibres pass transversely and slightly anteriorly inserting onto a mid-ventral aponeurosis.

Innervation

The innervation of the muscle is by a nerve arising medially from the r.mandibularis externus. Before Vc enters the lower jaw, it gives off a fairly thick branch which passes through a foramen in the surangular and proceeds anteriorly, lateral to the mandible, and so becomes the r.mandibularis externus. Before this nerve enters the foramen a branch is given off, the n.constrictor I ventralis (Fig. 67) which passes ventrally round the cartilage of Meckel, then medially over the dorso-medial margin of the gonial and then for a short distance anteriorly parallel to the mandible. During the course of this nerve anteriorly a number of radicles are given off which innervate the m.intermandibularis.

FACIAL MUSCULATURE

M.DEPRESSOR MANDIBULAE (Fig. 49)

The m.depressor mandibulae is single, but possesses an aponeurosis in the region of its insertion onto the processus retro-articularis and on the posterior concave surface of the articular. This aponeurosis develops relatively late in ontogeny but never completely separates the muscle into two sections. FIEDLER

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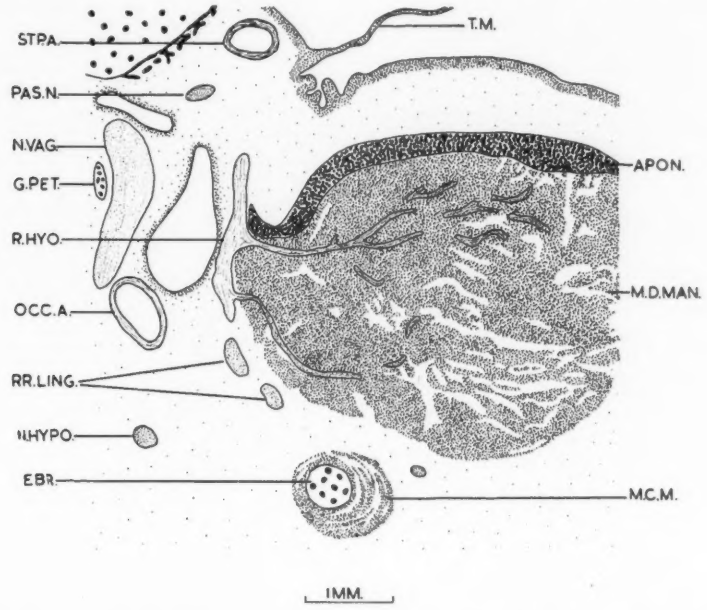

Fig. 56. Transverse section through the m.depressor mandibulae in the 37-day old embryo. *APON.*, aponeurosis; *EBR.*, epibranchial; *G.PET.*, g.petrosum; *M.C.M.*, m.ceratomandibularis; *M.D.MAN.*, m.depressor mandibulae; *N.HYPO.*, n.hypoglossus; *N. VAG.*, n.vagus; *OCC.A.*, occipital artery; *PAS.N.*, parasympathetic nerve; *R.HYO.*, r.hyomandibularis; *RR.LING.*, rami linguali; *STP.A.*, stapedial artery; *T.M.*, tympanic membrane.

(1951) states that the m.depressor mandibulae in *Oscines* is in two sections, but also states that these two sections are not clear.

The origin of the muscle in the late embryos is on the squamosal, exoccipital and supraoccipital, the fibres pass ventrally and slightly anteriorly. The plane in which the muscle lies in relation to the fibres varies with the age of the embryo. In the 13-day old embryo the muscle is horizontally placed, this position being gradually altererd to a practically vertical one in the 37-day old embryo and in the adult.

In the adult the muscle arises broadly on the posterior cranial wall, partially covering the squamosal and supraoccipital; the paroccipital process of the exoccipital is completely covered posteriorly by the muscle. The insertion is the same as described above.

Innervation

The muscle is innervated from branches of the r.hyomandibularis (Fig. 56).

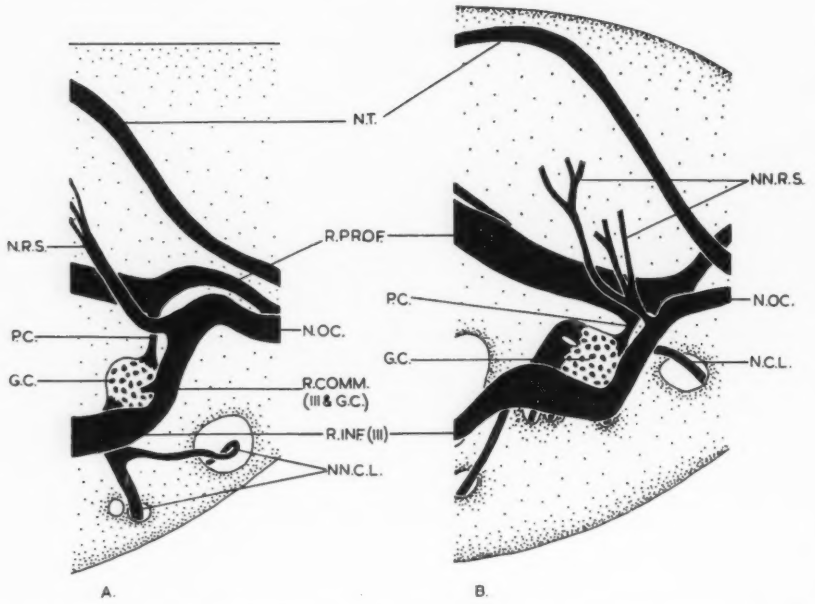

Fig. 57 A and B. Graphic reconstructions of the g.ciliare and associated nerves. A) 27-day old embryo. × 16·6. B) 37-day old embryo. × 10. *G.C.*, g.ciliare; *N.C.L.*, n.ciliaris longi; *NN.C.L.*, nervi ciliari longi; *NN.R.S.*, nerves to rectus superior; *N.OC.*, n.oculomotorius; *N.R.S.*, n.rectus superior; *N.T.*, n.trochlearis; *P.C.*, pars ciliaris; *R.COMM* (III and *G.C.*)., ramus communicans between n.oculomotorius and g.ciliare; *R.INF.*, ramus inferior of n.occulomotorius; *R.PROF.*, r.profundus.

THE CRANIAL PARASYMPATHETIC NERVOUS SYSTEM

The parasympathetic nerves, although basically constant, vary considerably in their relation to the cranial nerves in the embryos used in this investigation. For a description of the parasympathetic nervous system the condition found in the 27 and 37-day old embryos will be mainly used.

1. GANGLION CILIARE

The g.ciliare is situated medially and posteroventrally to the orbital capsule; it is relatively large and becomes connected to a number of the cranial nerves. The connexion of the ganglion to the r.profundus (Va) is, in all the embryos studied, by means of a short nerve, the pars ciliaris, passing more or less directly dorsally (Fig. 57 A and B). In one of the adult heads dissected the ganglion is not directly connected to the r.profundus by the pars ciliaris, but the ciliary nerves (nervi ciliari longi) from the ganglion have a connexion with the r.profundus (Fig. 58).

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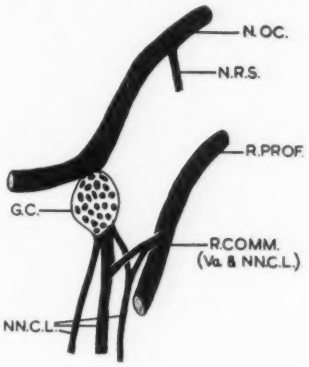

Fig. 58. Sketch illustrating the connexion between Va and the nervi ciliari longi in the adult. *G.C.*, g.ciliare; *NN.C.L.*, nervi ciliari longi; *N.OC.*, n.oculomotorius; *N.R.S.*, n.rectus superior; *R.COMM* (*Va* and *NN.C.L.*)., r.communicans between Va and nervi ciliari longi; *R.PROF.*, r.profundus.

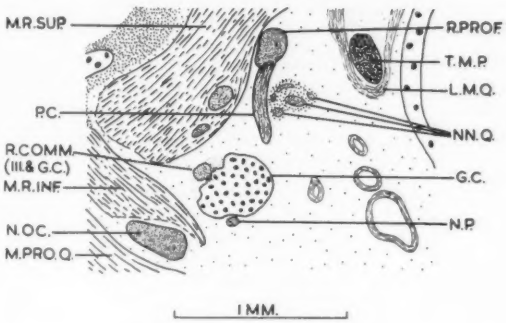

Fig. 59. Transverse section through the g.ciliare in the 27-day old embryo. *G.C.*, g.ciliare; *L.M.Q.*, loop of m.quadratus; *M.PRO.Q.*, m.protractor quadrati; *M.R.INF.*, m.rectus inferior; *M.R.SUP.*, m. rectus superior; *NN.Q.*, nervi quadrati; *N.OC.*, n.oculomotorius; *N.P.*, n.pyramidalis; *P.C.*, pars ciliaris; *R.COMM* (III and *G.C.*)., r.communicans between n.oculomotorius and g.ciliare; *R.PROF.*, r.profundus; *T.M.P.*, tendon of the m.pyramidalis.

The g.ciliare is also connected to the n.oculomotorius by means of a short nerve (Fig. 57 A) which arises inside the ganglion; after leaving the ganglion it comes to lie very close to its medial surface (Fig. 59). Apart from this connexion between the g.ciliare and the n.oculomotorius, there are small subsidiary connexions between them when the nerve passes very close to the medial surface of the ganglion.

The connexions between the g.ciliare and the r. profundus and n.oculomotorius are constant in all the embryos studied. In the 13-day old embryo there is in addition, a small nerve connecting the g.ciliare with the n.abducens (Fig. 60). None of the other embryos studied shows this.

The nervi ciliari longi leaving the ganglion vary considerably in number. In the 27-day old embryo there is a single nerve which further divides into two radicles, a larger and a smaller (Fig. 57 A); they traverse the cartilaginous orbital capsule. In the 37-day old embryo there are five ciliary nerves all leaving the ganglion more or less independently and each passing through the cartilaginous orbital capsule (Fig. 57 B). These nerves all pass along the floor of the orbit and innervate the ciliary muscles of the eye (Fig. 61). The innervation

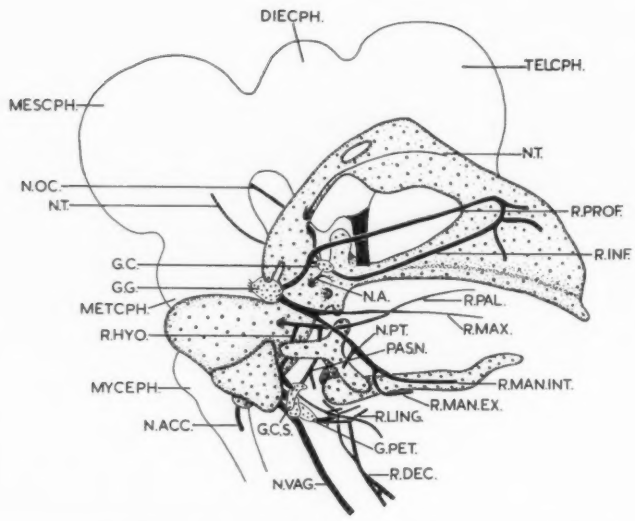

Fig. 60. Graphic reconstruction of the chondrocranium and nerves in the 13-day old embryo. × 6·7. Lateral aspect. *DIECPH.*, diencephalon; *G.C.*, g.ciliare; *G.C.S.*, g.cervicale supremum; *G.G.*, g.gasseri; *G.PET.*, g.petrosum; *MESCPH.*, mesencephalon; *METCPH.*, metencephalon; *MYCEPH.*, myelencephalon; *N.A.*, n.abducens; *N.ACC.*, n.accessorius; *N.OC.*, n.oculomotorius; *N.PT.*, n.pterygoideus; *N.T.*, n.trochlearis; *N.VAG.*, n.vagus; *PAS.N.*, parasympathetic nerve; *R.DEC.*, r.descendens; *R.HYO.*, r.hyomandibularis; *R.INF.*, r.inferior (III); *R.LING.*, r.lingualis; *R.MAN.EX.*, r.mandibularis externus; *R.MAN.INT.*, r.mandibularis internus; *R.MAX.*, r.maxillaris; *R.PAL.*, r.palatinus; *R.PROF.*, r.profundus; *TELCPH.*, telencephalon.

of the dorsal part of the ciliary muscle is by two nerves that pass anteriorly and posteriorly to the lens.

2. GANGLION SPHENOPALATINUM

This is a very small ganglion situated anteriorly in the orbital region between the septum and the m.rectus anterior (Figs. 62 and 63). From it pass two radicles: one in an antero-ventral and the other in an antero-dorsal direction. The antero-ventral radicle is short and innervates the Harderian gland (Fig. 63). The antero-dorsal branch divides into three smaller radicles, two of which appear to end abruptly, while the third and largest joins with a branch of Va (Fig. 63). This connexion of Va with the parasympathetic nervous system is very clearly seen in the 15-day old embryo (Fig. 64). In the 27-day old embryo, just before the connexion of the parasympathetic nerve with Va is effected, a small radicle is given off that innervates the nasal glands (Fig. 63).

Extending posteriorly from the g.sphenopalatinum is a thick nerve, the n.sphenopalatinus, which lies medial to the orbit and close to the interorbital septum. During its posterior course it passes slightly ventrally and dorsally over the processus basipterygoideus (Fig. 63). Posterior to this it comes to

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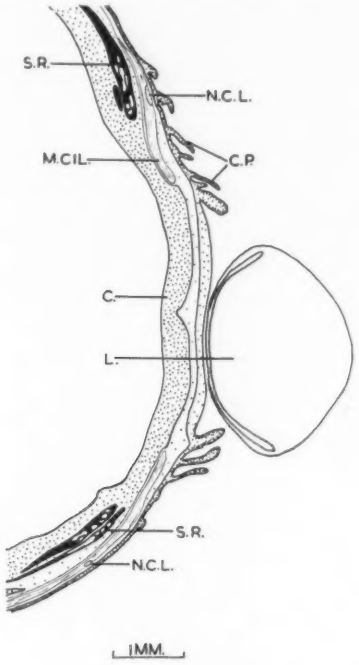

Fig. 61. Transverse section of part of the eye, illustrating the innervation of the ciliary muscles in the 37-day old embryo. *C.*, cornea; *C.P.*, ciliary process; *L.*, lens; *M.CIL.*, m.ciliaris; *N.C.L.*, n. ciliaris longi; *S.R.*, scleral ring.

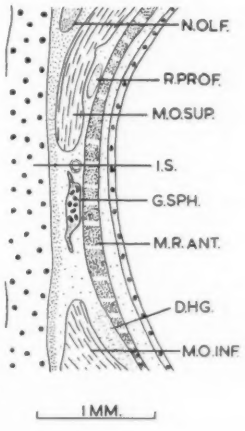

Fig. 62. Transverse section through the g.sphenopalatinum in the 27-day old embryo. *D.H.G.*, duct of Harderian gland; *G.SPH.*, g.sphenopalatinum; *I.S.*, interorbital septum; *M.O.INF.*, m.obliquus inferior; *M.O.SUP.*, m.obliquus superior; *M.R.ANT.*, m. rectus anterior; *N.OLF.*, n.olfactorius; *R.PROF.*, r.profundus.

lie close to the r.palatinus (VII). In the 27-day old embryo the Vidian or parabasal canal, through which the r.palatinus and parasympathetic nerve is going to pass, has not yet formed, while in the 37-day old embryo they do pass through the completed canal (Fig. 65 and Fig. 27). In the 27-day old embryo the n.sphenopalatinus lies medial to the r. palatinus (VII), and in places it is very difficult to distinguish between the two nerves (Fig. 66).

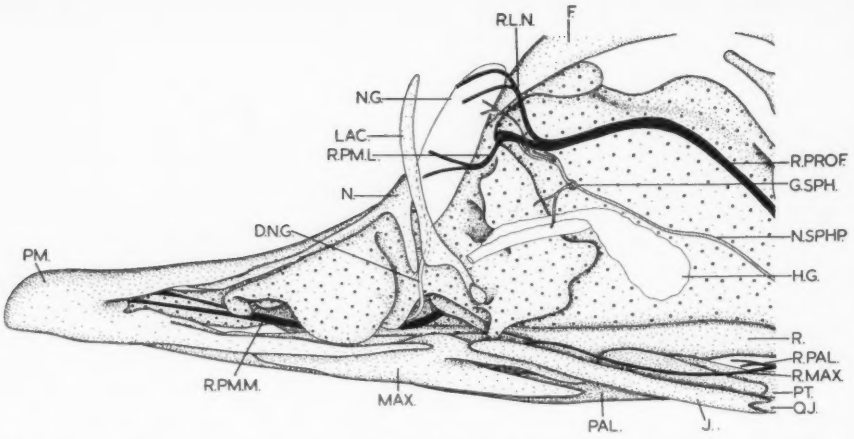

Fig. 63. Graphic reconstruction of the anterior part of the skull in the 27-day old embryo. × 5. Lateral aspect. *D.N.G.*, duct of nasal gland; *F.*, frontal; *G.SPH.*, g.sphenopalatinum; *H.G.*, Harderian gland; *J.*, jugal; *LAC.*, lacrimal; *MAX.*, maxillary; *N.*, nasal; *N.G.*, nasal gland; *N.SPHP.*, n.sphenopalatinus; *PAL.*, palatine; *P.M.*, premaxillary; *P.T.*, pterygoid; *QJ.*, quadratojugal; *R.*, rostrum; *R.L.N.*, r.lateralis nasi; *R.MAX.*, r.maxillaris; *R.PAL.*, r.palatinus; *R.PM.L.*, r.premaxillaris lateralis; *R.PM.M.*, r.premaxillaris medialis; *R.PROF.*, r.profundus.

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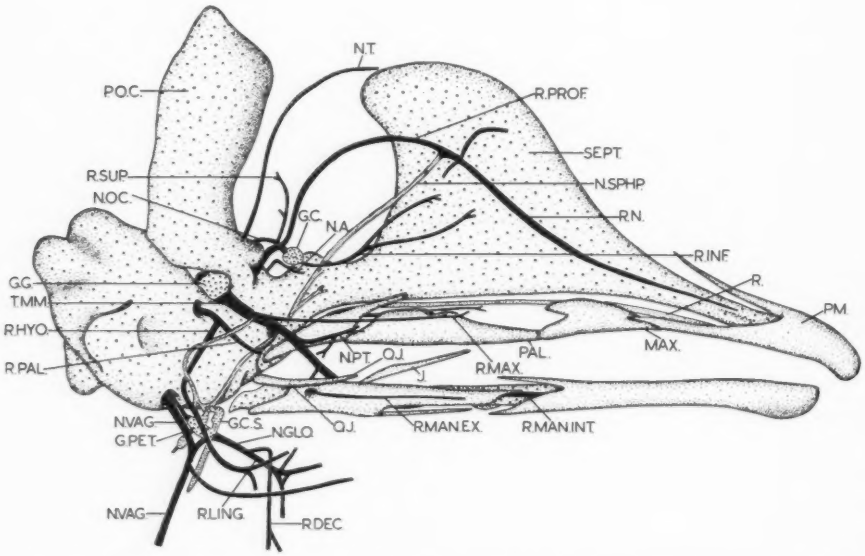

Fig. 64. Contour reconstruction of the nerves and chondrocranium in the 15-day old embryo. × 8·3. Lateral aspect. *G.C.*, g.ciliare; *G.C.S.*, g.cervicale supremum; *G.G.*, g.gasseri; *G.PET.*, g.petrosum; *J.*, jugal; *MAX.*, maxillary; *N.A.*, n.abducens; *N.GLO.*, n.glossopharyngeus; *N.OC.*, n.oculomotorius; *N.PT.*, n.pterygoideus; *N.SPHP.*, n.sphenopalatinus; *N.T.*, n.trochlearis; *N.VAG.*, n.vagus; *PAL.*, palatine; *P.M.*, premaxillary; *P.O.C.*, post-orbital cartilage; *QJ.*, quadratojugal; *R.*, rostrum; *R.DEC.*, r.descendens; *R.HYO.*, r.hyomandibularis; *R.INF.*, r.inferior (III); *R.LING.*, r.lingualis; *R.MAN.EX.*, r.mandibularis externus; *R.MAN.INT.*, r.mandibularis internus; *R.MAX.*, r.maxillaris; *R.N.*, r.nasalis; *R.PAL.*, r.palatinus; *R.PROF.*, r.profundus; *R.SUP.*, r.superior (III); *SEPT.*, septum (nasal and interorbital); *T.M.M.*, truncus maxillo-mandibularis.

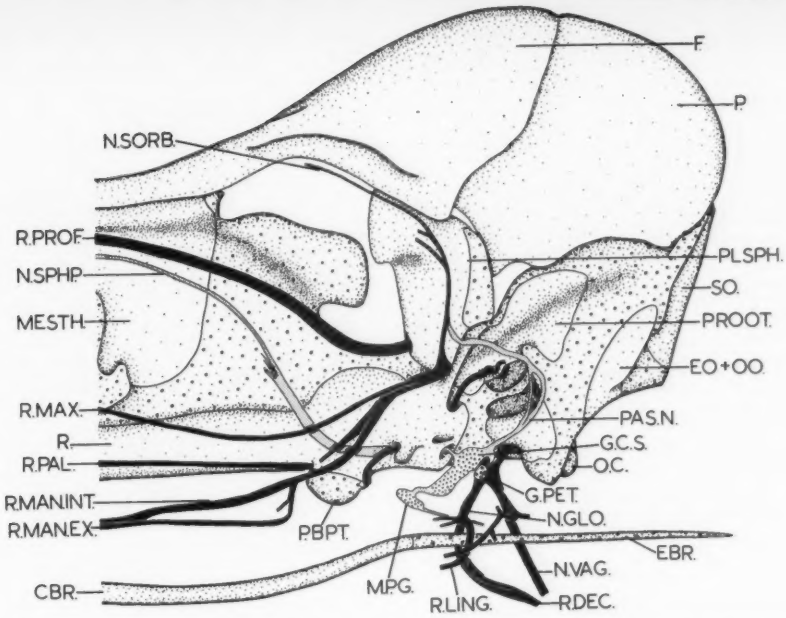

Fig. 65. Graphic reconstruction of the posterior region of the skull and nerves in the 37-day old embryo. × 3·3. Lateral aspect. *CBR.*, ceratobranchial; *EBR.*, epibranchial; *EO + OO.*, exoccipital + opisthotic; *F.*, frontal; *G.C.S.*, g.cervicale supremum; *G.PET.*, g.petrosum; *MESTH.*, mesethmoid; *M.P.G.*, median parasympathetic ganglion; *N.GLO.*, n.glossopharyngeus; *N.SORB.*, n.supraorbitalis; *N.SPHP.*, n.sphenopalatinus; *N.VAG.*, n.vagus; *O. C.*, occipital condyle; *P.*, parietal; *PAS.N.*, parasympathetic nerve; *P.BPT.*, processus basipterygoideus; *PLSPH.*, pleurosphenoid; *PROOT.*, prootic; *R.*, rostrum; *R.DEC.*, r.descendens; *R.LING.*, r.lingualis; *R.MAN.EX.*, r.mandibularis externus; *R.MAN.INT.*, r.mandibularis internus; *R.MAX.*, r.maxillaris; *R.PAL.*, r.palatinus; *R.PROF.*, r.profundus; *SO.*, supraoccipital.

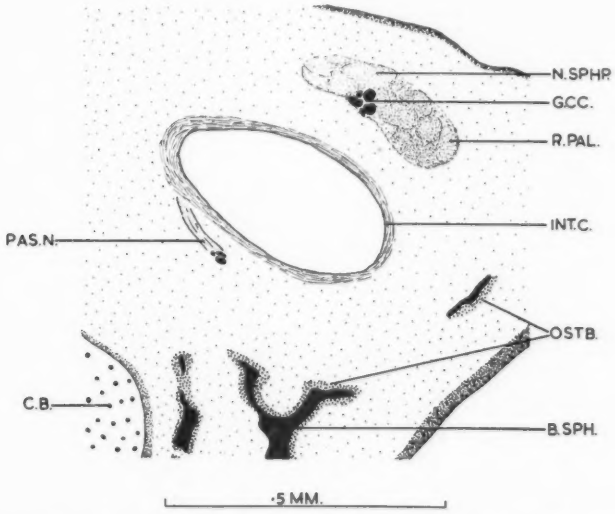

Fig. 66. Transverse section illustrating the connexion between the r.palatinus and n.sphenopalatinus in the 27-day old embryo. *BSPH.*, basisphenoid; *C.B.*, cranial base; *G.C.C.*, ganglion cells; *INT.C.*, internal carotid; *N.SPHP.*, n.sphenopalatinus; *OSTB.*, osteoblasts; *PAS.N.*, parasympathetic nerve; *R.PAL.*, r.palatinus.

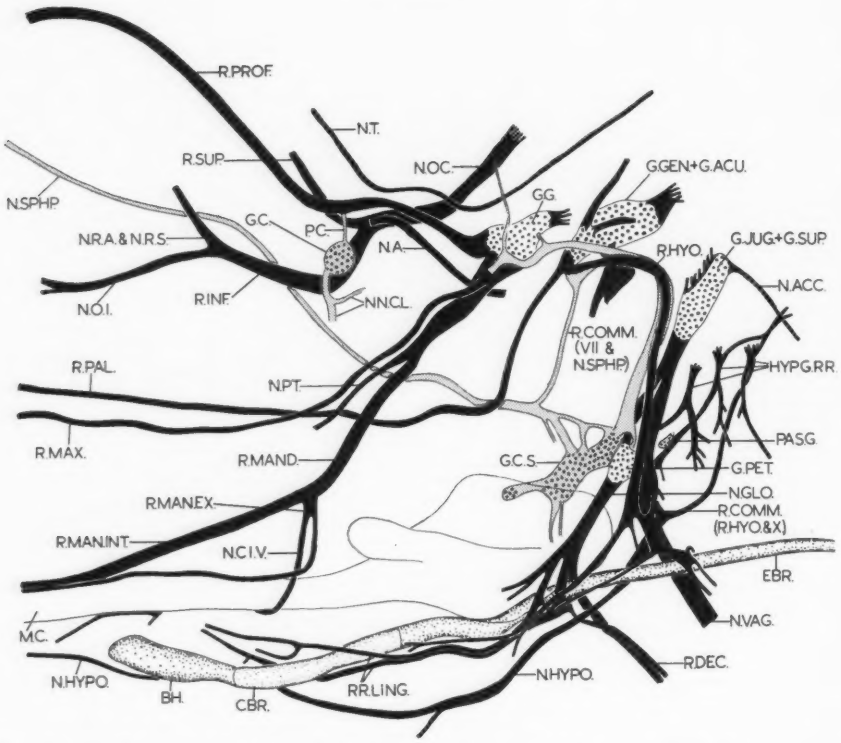

Fig. 67. Graphic reconstruction of the peripheral and cranial parasympathetic nerves of the 27-day old embryo (posterior part). × 8·3. Lateral aspect. *BH.*, basihyale; *CBR.*, ceratobranchial; *EBR.*, epibranchial; *G.C.*, g.ciliare; *G.C.S.*, g.cerviale supremum; *G.EN.+G.ACU.*, g.geniculatum + g.accusticus; *G.G.*, g.gasseri; *G.PET.*, g.petrosum; *G.JUG. + G.SUP.*, g.jugulare + g.superius; *HYPG.RR.*, hypoglossal roots; *M.C.*, Meckel's cartilage; *N.A.*, n.abducens; *N.ACC.*, n.accessorius; *N.CI.V.*, n.constrictor I ventralis; *N.GLO.*, n.glossopharyngeus; *N.HYPO.*, n.hypoglossus; *NNC.L.*, nervi ciliari longi; *N.OC.*, n.oculomotorius; *N.O.I.*, n.obliquus inferior; *N.PT.*, n.pterygoideus; *N.R.A. + N.R.S.* n.rectus anterior + n.rectus superior; *N.SPHP.*, n.sphenopalatimus; *N.T.*, n.trochlearis; *N.VAG.*, n.vagus; *PAS.G.*, parasympathetic ganglion; *P.C.*, pars ciliaris; *R.COMM.* (VII and *N.SPHP.*)., r.communicans between the n.facialis and n.sphenopalatinus; *R.COMM.* (*R.HYO.* and *X*)., r.communicans between r.hyomandibularis and n.vagus; *R.DEC.*, r.descendens; *R.HYO.*, r.hyomandibularis; *R.INF.*, r.inferior (III); *R.MAND.*, r.mandibularis; *R.MAN.EX.*, r.mandibularis externus; *R.MAN.INT.*, r.mandibularis internus; *R.MAX.*, r.maxillaris; *R.PAL.*, r.palatinus; *R.PROF.*, r.profundus; *RR.LING.*, rami linguali; *R.SUP.*, r.superior (III).

Where they lie close together there are small groups of ganglion cells (Fig. 66), together with nerve fibres passing from the n.sphenopalatinus to the r.palatinus. These connexions are also present in the 15 and 37-day old embryos.

Further back, in the 27-day old embryo, the n. sphenopalatinus gives off a radicle that passes dorsally over the lateral surface of the prootic region of the auditory capsule and forms a connexion with the r.hyomandibularis (VII) as it leaves the foramen faciale (Figs. 67 and 68). In the 15-day old embryo

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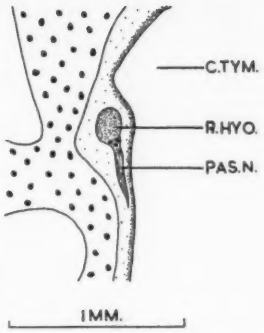

Fig. 68. Transverse section illustrating the connexion between the r.hyomandibularis and a parasympathetic nerve in the 27-day old embryo. *C.TYM.*, cavum tympanicum; *PAS.N.*, parasympathetic nerve; *R.HYO.*, r.hyomandibularis.

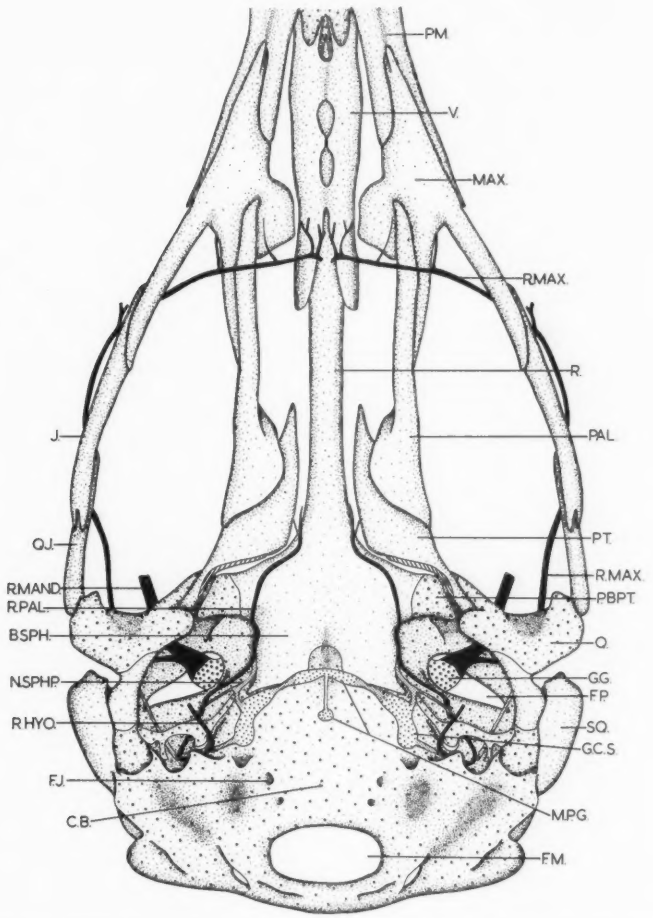

Fig. 69. Graphic reconstruction of the skull and nerves in the 27-day old embryo. × 5. Ventral aspect. *BSPH.*, basisphenoid; *C.B.*, cranial base; *F.J.*, foramen jugulare; *F.M.*, foramen magnum; *F.P.*, foramen profundum; *G.C.S.*, g.cervicale supremum; *G.G.*, g.gasseri; *J.*, jugal; *MAX.*, maxillary; *M.P.G.*, median parasympathetic ganglion; *N.SPHP.*, n.sphenopalatinus; *PAL.*, palatine; *P.BPT.*, processus basipterygoideus; *P.M.*, premaxillary; *P.T.*, pterygoid; *Q.*, quadrate; *QJ.*, quadratojugal; *R.*, rostrum; *R.HYO.*, r.hyomandibularis; *R.MAND.*, r.mandibularis; *R.MAX.*, r.maxillaris; *R.PAL.*, r.palatinus; *SQ.*, squamosal; *V.*, vomer.

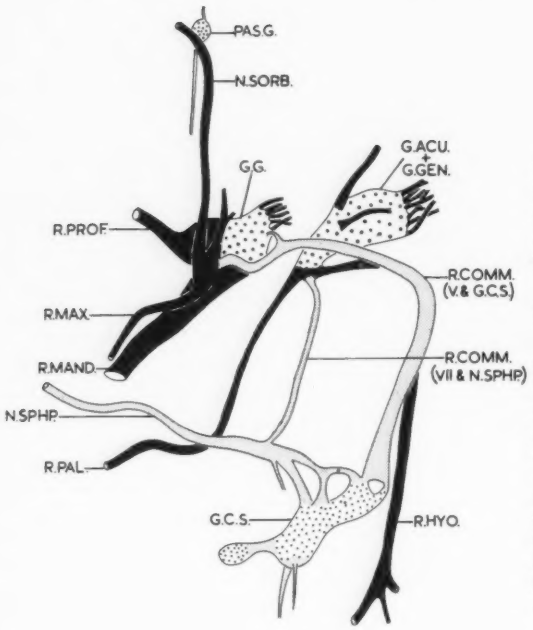

Fig. 70. Graphic reconstruction of the posterior parasympathetic nerves and their relation to the peripheral cranial nerves numbers V and VII in the 27-day old embryo. × 11·1. Lateral aspect. *G.ACU.* + *G.GEN.*, g.acusticus + g.geniculatum; *G.C.S.*, g.cervicale supremum; *G.G.*, g.gasseri; *N.SORB.*, n.supraorbitalis; *N.SPHP.*, n.sphenopalatinus; *PAS.G.*, parasympathetic ganglion; *R.COMM.* (*V* and *G.C.S.*)., r.communicans between r.maxillaris and g.cervicale supremum; *R.COMM.* (*VII* and *N.SPHP.*)., r.communicans between r.palatinus and n.sphenopalatinus; *R.HYO.*, r.hyomandibularis; *R.MAND.*, r.mandibularis; *R.MAX.*, r.maxillaris; *R.PAL.*, r.palatinus; *R.PROF.*, r.profundus.

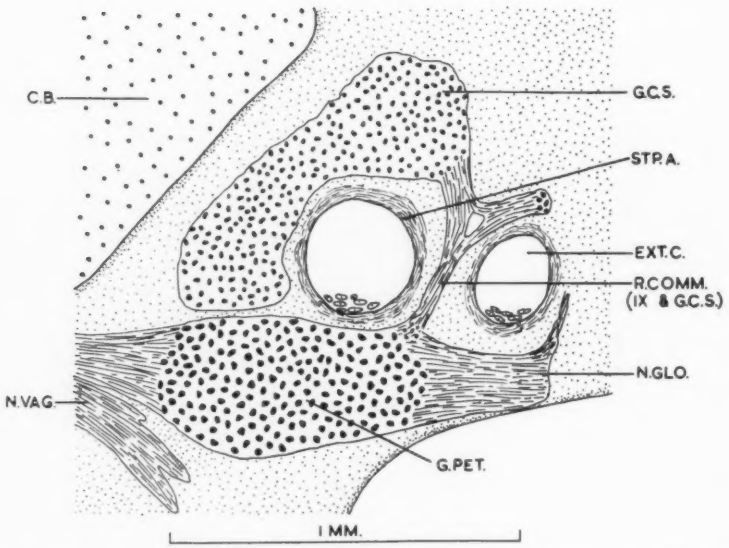

Fig. 71. Sagittal section of the g.cervicale supremum and g. petrosum in the 15-day old embryo. *C.B.*, cranial base; *EXT.C.*, external carotid; *G.C.S.*, g.cervicale supremum; *G.PET.*, g.petrosum; *N.GLO.*, n.glossopharyngeus; *N.VAG.*, n.vagus; *R.COMM.* (*IX* and *G.C.S.*)., r.communicans between n.glosspharyngeus and g.cervicale supremum; *STP.A.*, stapedial artery.

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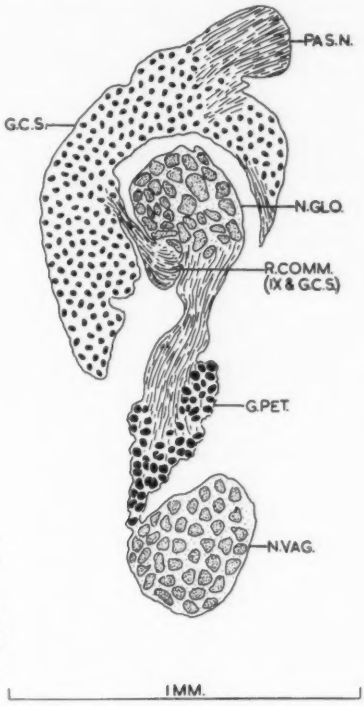

Fig. 72. Transverse section of the g.cervicale supremum; illustrating its connexion with the n.glossopharyngeus in the 37-day old embryo.
G.C.S. g.cervicale supremum; *G.PET.*, g.petrosum; *N.GLO.*, n.glossopharyngeus; *N.VAG.*, n.vagus; *PA.S.N.*, parasympathetic nerve; *R.COMM.* (*IX* and *G.C.S.*), r.communicans between n.glossopharyngeus and g.cervicale supremum.

there is a parasympathetic connexion with the r.hyomandibularis some distance along its length (Fig. 64). In the 21-day old embryo there is a connexion between the parasympathetic nervous system and the r.palatinus immediately before the r. palatinus enters the foramen faciale. In the 37-day old embryo there is no connexion of the r.hyomandibularis with the n.sphenopalatinus. It appears that the connexion of the r.hyomandibularis is embryonic only, with the point of connexion variable.

Posterior to the point where the parasympathetic nerve connecting with the r.hyomandibularis is given off, there are, in the 27-day old embryo (Fig. 67), three connexions with the ventro-laterally situated *g.cervicale supremum*. In the 37-day old embryo there is a single nerve, connecting with the ganglion anteriorly (Fig. 65). In the 27-day old embryo the cervical ganglia of either side are connected by a nerve to a transversly elongated medially placed ganglion (Fig. 69), from which there extends posteriorly a short nerve which terminates in a small third ganglion. In the 37-day old embryo this latter ganglion is lost and the connecting nerve ends abruptly (Fig. 65).

From the g.cervicale supremum there passes dorsally a large parasympathetic nerve, posterior to the tympanic cavity. Dorsal to the tympanic cavity it passes anteriorly to become connected with the r.maxillaris. This connexion with the

r.maxillaris is seen in both the 15 and 27-day old embryos (Figs. 64 and 67). In the 37-day old embryo, this parasympathetic nerve is partially double (Fig. 65) and does not connect with the r.maxillaris, but with the r.supraorbitalis which arises from the truncus maxillo-mandibularis (Fig. 65).

Posteriorly, at the point where the above parasympathetic nerve is given off from the g. cervicale supremum, a small window is formed in the ganglion through which passes the n.glossopharyngeus (Figs. 65, 67 and 70). As it passes through the window a connexion is effected with the ganglion; this is clearly visible in the 15, 27 and 37-day stages (Figs. 71 and 72).

THE PERIPHERAL CRANIAL NERVES

The cranial nerves in the various stages studied follow a more or less constant course. However, their connexions with the parasympathetic nervous system vary. For an account of the cranial nerves of *Struthio*, the 27-day old embryo will be used as a basis, as it is in this stage that the nerves are most easily distinguished. The other developmental stages will be referred to where variations actually occur.

Nerves I and II will not be discussed, and nerves III, IV and VI have previously been dealt with in discussing the eye musculature.

A. N.TRIGEMINUS

The g.gasseri (G.semilunare, STRESEMANN, 1927—1934) is large and incompletely divided into dorsal and ventral sections. This is very clearly seen in all the stages (Figs. 64, 67 and 70). From the dorsal section of the ganglion arises the r.profundus (Va) and v. HALLERSTEIN (1934) refers to this part of the ganglion, as the g.ophthalmicum. In *Boulengerula*, DE VILLIERS (1938) states that the ganglion of V is divided into two, though having a common root, the g.ophthalmicum Va and the g.maxillo-mandibulare (Vb and c). In *Scolecomorphus* the gasserian ganglion is not a bipartite mass, but a single ganglion from which Va, Vb and Vc take their origin (DE VILLIERS, 1938). From the ventral section of the ganglion arises the truncus maxillo-mandibularis, and this part of the ganglion is known as the g.maxillo-mandibularis. The n.trigeminus is divided into a major sensory portion which is dorsal and lateral, and a minor portion which is ventral and medial (STRESEMANN, 1927—1934). The sensory portion comprises two branches: (1) the r.ophthalmicus profundus (or Waldschmidt's nerve), the so-called Va; (2) the truncus maxillo-mandibularis which has a dorsal branch, the r. maxillaris (Vb) and a ventral branch, the r. mandibularis (Vc). The motor portion of the trigeminus is a branch of Vc—the n.pterygoideus. It has already been dealt with.

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(1) *Ramus Profundus* (Va)

The r.profundus, after leaving the ganglion, passes forwards medial to the pleurosphenoid for a short distance and then enters the foramen profundum, which has its internal opening between the pleurosphenoid and basisphenoid. The foramen is diagonally disposed in the transverse plane and is bounded for its whole length by the pleurosphenoid above and the basisphenoid below. The external opening of the foramen is on the antero-medial border of the pleurosphenoid where the latter meets the basisphenoid (Fig. 65). The nerve, after leaving the foramen, passes anteriorly and dorsally, and lies close to the interorbital septum. Soon after it has left the foramen, it is joined to the g.ciliare, as previously described.

During its course anteriorly, the r.profundus lies lateral to the m.rectus superior and lateral to the m.rectus anterior, passing dorsally over this muscle to lie between it and the interorbital septum. More anteriorly the nerve lies between the m.rectus anterior and m.obliquus superior. Between these two muscles the r.profundus divides into a small dorsal ramus, and a larger ventral ramus. The dorsal ramus, the r.lateralis nasi (Cords, 1904), passes dorsally and comes to lie lateral to the frontal and divides into a number of radicles which innervate the dermal epithelium on the latero-dorsal surfaces of the anterior facial region. The ventral ramus divides into two branches at the point of the origin of the m.rectus anterior and the m.obliquus superior and dorsal to the planum antorbitale. These two branches are: the r.praemaxillaris superior (lateralis) and the r.praemaxillaris inferior (medialis) (v. Hallerstein, 1934).

The r.praemaxillaris superior (lateralis) is a small ventral branch, which is directly connected to the g.sphenopalatinum (Fig. 63), and which passes anteriorly between the glandes nasales and the os nasale to innervate the dorsal dermal epithelium of the beak. The r.praemaxillaris inferior (medialis) which is the larger dorsal and more median branch, passes anteriorly for a short distance, through the foramen olfactorium advehens, and then ventrally along the septum nasi, and finally follows an anterior course lateral to the fused trabeculae and rostrum. It then divides into two branches which terminate in numerous fine radicles which innervate the anterior part of the beak (Fig. 63).

Discussion

Brock (1937) states that the ethmoidal nerve, which, in fact, is the r.profundus, has a strong r.medialis (r.praemaxillaris inferior or medialis of v. Hallerstein, 1934), which passes forwards inside the capsule against the nasal septum and continues in front of the capsule along the prenasal process. Brock (1937) further states that the r.lateralis (r.praemaxillaris superior or lateralis of v. Hallerstein) is reduced to a filament directed towards the

concha; this is not the case in the Snipe (*Scolopax*) where it is a very large nerve and lies in a bony groove on the dorsal side of the premaxillary (v. HALLERSTEIN, 1934).

(2) *Ramus Maxillaris* (Vb)

The second branch of the major pretrematic portion of the n.trigemius is the truncus maxillo-mandibularis, which, though mainly sensory in nature, contains motor fibres which go to the trigeminus musculature. The truncus maxillo-mandibularis arises from the ventral portion of the g.gasseri; the g.maxillo-mandibularis (v. HALLERSTEIN, 1934). The truncus maxillo-mandibularis is a short, thick nerve from which the r.maxillaris (Vb) and the r. mandibularis (Vc) are given off.

The r.maxillaris passes laterally and antero-ventrally beneath the orbit, and it also passes above the quadratojugal and jugal. Where the latter meets the zygomatic process of the maxillary, the r.maxillaris passes rostrally and lies ventral to the maxillo-palatine arthrosis and the posterior end of the vomer. From the r.maxillaris, during its rostral course, are given off a number of small radicles which innervate the epithelium of the anterior palate (Fig. 69).

In the 37-day old embryo the r.maxillaris divides soon after it is given off from the truncus maxillo-mandibularis into two branches: a lateral one which is the larger, and comes to lie dorsal to the m.depressor palpebrae inferioris, and a medial one which is much smaller and lies ventral to the m.depressor palpebrae inferioris (Fig. 73). The medial branch passes anteriorly lateral to the pterygoid and palatine, and innervates the mucous epithelium of the posterior palate immediately ventral to the pterygoid.

The lateral branch of Vb follows a lateral course ventral to the bulbus and lies immediately dorsal to the quadratojugal and jugal, in which region are given off numerous radicles that innervate the lateral margins of the upper jaw and the lower eyelid. The nerve continues anteriorly, but moves from a position dorsal to the lower temporal bar and comes to lie medial to the jugal. In the region of the anterior part of the jugal it passes rostrally ventral to the lacrimal and innervates, after branching into numerous radicles, the epithelium of the palate in the region of the vomer.

V. HALLERSTEIN (1934) states that during the course of the main branch of the r.maxillaris rostrally, it passes through a bony canal. This is not the case for *Struthio* as at no time does the nerve pass through a foramen. Further, he mentions that in *Gallus domesticus* the r.maxillaris is connected by a thin branch to the r.palatinus (VII) which is homologous with the r.communicans anterior of reptiles. In none of the embryos studied does this connexion occur in *Struthio*.

V. HALLERSTEIN (1934) states for *Gallus* that the r.maxillaris is divided into a lateral division and a main branch. In the 37-day stage of *Struthio* as has

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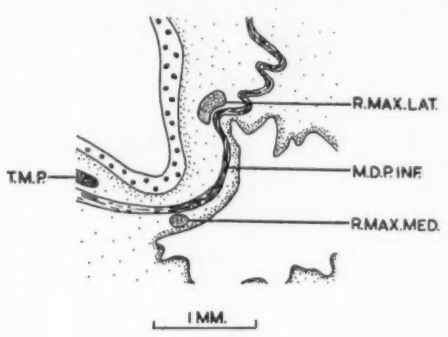

Fig. 73. Transverse section illustrating the r.maxillaris lateralis and r. maxillaris medialis in the 37-day old embryo. *M.D.P.INF.*, m.depressor palpebrae inferioris; *R.MAX.LAT.*, r.maxillaris lateralis; *R.MAX.MED.*, r.maxillaris medialis; *T.M.P.*, tendon of m.pyramidalis.

been shown, there are two divisions, of which the lateral one is the main branch and the medial very much smaller. It is not certain whether these two branches in the 37-day old embryo are in any way homologous with the two branches of the r.maxillaris referred to by v. HALLERSTEIN (1934). It can, however, be stated that the main branch of the r.maxillaris innervates the margin of the jaw, and so corresponds to the main branch of Vb in the fowl (v. HALLERSTEIN, 1934). The medial branch of Vb in *Struthio* has no counterpart in *Gallus*.

3. *Ramus Mandibularis* (Vc)

This nerve possesses both somatic sensory and visceral motor fibres. The visceral motor fibres of Vc have previously been dealt with in the section dealing with the trigeminus musculature.

The somatic sensory fibres of Vc are embodied in the r.mandibularis after the n.pterygoideus has been given off except for the motor fibres that innervate the m.intermandibularis. The r.mandibularis passes ventrally and gives off a branch, the r.mandibularis externus, which also divides into two branches. There is a small inner branch, which has both motor and sensory fibres, and passes ventrally round Meckel's cartilage, and gives off numerous radicles which innervate the innerside of the lower jaw and the m.intermandibularis. The outer branch of the r. mandibularis externus passes ventrally and comes to lie lateral to the lower jaw, having passed through a foramen in the surangular. The r.mandibularis externus passes anteriorly along the lateral surface of the surangular and extends no farther forwards than the anterior end of the bone, in which region it breaks up into numerous radicles which innervate the skin of the lateral margin of the lower jaw.

The main branch of the r.mandibularis is the r.mandibularis internus, which, after having been separated from the r.mandibularis externus, follows an anterior course medial to the surangular and latero-dorsal to Meckel's cartilage; more anteriorly the r.mandibularis internus becomes covered dorsally by the

medio-dorsal posterior process of the dentary, and still farther forwards this nerve is more or less lateral to Meckel's cartilage and medial to the dentary. It retains this position up to the anterior end of the dentary, at which point it enters the bone and breaks up into numerous radicles which pass through their own foramina and come to innervate the skin of the lower jaw anteriorly.

V. HALLERSTEIN (1934) states that joined to the r.mandibularis internus there is a small ganglion, the g.submandibulare. No such ganglion is present in *Struthio*. GAUPP, according to v. HALLERSTEIN (1934), observed in reptiles a knot at the junction of the r.mandibularis and the chorda tympani (VII). It is quite probable that v. HALLERSTEIN (1934) assumed that a ganglion is present here, as he describes a chorda tympani in *Gallus*, though there is no such nerve in *Struthio* or in *Spheniscus* (FRANK, 1954).

STRESEMANN (1927—1934) maintains that all three branches of the n.trigeminus have facial connexions. In *Struthio* no such connexions could be found.

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B. N. FACIALIS (VII)

The g.geniculare is small, and from it a single nerve passes laterally in the foramen facialis. As the nerve leaves the foramen it divides into two main branches: the r.palatinus (VII) and the r.hyomandibularis (VII).

1. *R. Palatinus*

The r.palatinus passes ventrally and slightly anteriorly over the surface of the prootic region of the auditory capsule and basisphenoid. It then follows an anterior course passing ventral to the processus basipterygoideus (Figs. 47, 65 and 69). In the 27-day stage the r.palatinus is short and soon after passing the processus basipterygoideus, it becomes divided into a number of small radicles which innervate the mucous epithelium of the posterior palate in the region of the rostral flanges of the pterygoid and the basisphenoid where it narrows to form the rostrum. In the 37-day stage the nerve is larger and lies dorsal to the rostral flange of the pterygoid (Fig. 54) after passing ventral to the processus basipterygoideus. In the 27-day old embryo the r.palatinus not only lies dorsal to the rostral flange of the pterygoid, but also lateral to it. The nerve gives off radicles that innervate the epithelium covering the posterior part of the pterygoid dorsally. The r.palatinus extends farther anteriorly along the dorso-lateral margin of the pterygoid and then lateral to the rostrum, and finally lies lateral to the posterior processes of the vomer. Throughout its course from the processus basipterygoideus, it gives off radicles that innervate the epithelium of the posterior portion of the palate.

As has already been seen, the r.palatinus in the 37-day old embryo passes through the Vidian or parabasal canal in which there is an exchange of parasympathetic nerves from the n.sphenopalatinus, coming from the g.cervicale

supremum. The canal is also present in *Gallus,* in which there is also an exchange of parasympathetic nerves (v. HALLERSTEIN, 1934). STRESEMANN (1927—1934) states that the r.anterior (r.palatinus) exchanges fibres with the n. glossopharyngeus and with the parasympathetic nervous system, and further states that round this region of exchange of fibres, is formed the g.ethmoidale. There is no ganglion in this region in *Struthio* though in the 27-day old embryo transitory ganglion cells were observed where the n.sphenopalatinus and r.palatinus join. There is also no exchange between the n.glossopharyngeus and the r.palatinus in *Struthio.*

2. *Ramus Hyomandibularis VII*

The r.hyomandibularis possesses both visceral motor and visceral sensory fibres. It leaves the foramen facialis as a large nerve passing posteriorly dorsal to the cavum tympanicum and then ventrally immediately posterior to the tympanic membrane. It follows a course similar to that of the parasympathetic nerve that leaves the g.cervicale supremum posteriorly. There is a very definite exchange of fibres beetwen the r.hyomandibularis and the parasympathetic nerve.

From the r.hyomandibularis, as it passes the m.depressor mandibulae, pass off branches that innervate the muscle (Fig. 56). The r.hyomandibularis continues ventrally and slightly anteriorly as a small branch that lies lateral to the ceratohyale, but does not extend very far forwards before breaking up into a number of radicles which innervate the muscles of the posterior hyoid musculature, the m.mylohyoideus posterior.

From the r.hyomandibularis, there is a very short thick nerve that passes postero-ventrally and from which a dorsal branch is given off that forms a connexion with the n.vagus (Fig. 67). There are also small branches that are given off postero-ventrally and antero-ventrally which go to the pharynx.

STRESEMANN (1927—1934) and v. HALLERSTEIN (1934) both describe a chorda tympani for *Gallus,* and BROCK (1937, p. 236) states: "The chorda tympani of the Ostrich in the latest stage, stage 4, branches from the hyomandibular above the columella shaft and its course is entirely precolumellar." The nerve that BROCK (1937) has called the chorda tympani is, in fact, a parasympathetic nerve that connects the n.sphenopalatinus with the r.hyomandibularis, and which is lost in the 37-day old embryo; *there is no chorda tympani in Struthio.*

C. N. GLOSSOPHARYNGEUS (IX)

The ganglion petrosum is ventro-laterally situated and lies lateral to the posterior part of the g.cervicale supremum to which it becomes connected. A large nerve leaves the g.petrosum and passes ventrally and soon divides into two main branches: a lateral and a medial (Fig. 67).

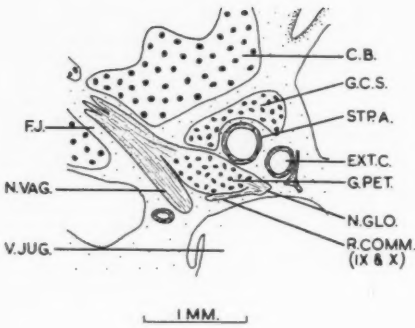

Fig. 74. Sagittal section to illustrate the connexion between the n.glossopharyngeus and the n.vagus in the 15-day old embryo. *C.B.*, cranial base; *EXT.C.*, external carotid; *F.J.*, foramen jugulare; *G.C.S.*, g.cervicale supremum; *G.PET.*, g.petrosum; *N.GLO.*, n.glossopharyngeus; *N.VAG.*, n. vagus; *R.COMM.* (*IX* and *X*); r.communicans between n.glossopharyngeus and n.vagus; *STP.A.*, stapedial artery; *V.JUG.*, vena jugularis.

The lateral branch divides into two, both of which lie lateral to the ceratohyale and become joined by a short r.communicans (Fig. 67). Both these nerves then pass anteriorly ventral to the ceratohyale, and are in fact the r.lingualis of IX; they innervate the m.ceratomandibularis and the posterior ventral region of the buccal cavity and tongue.

The medial branch of the n.glossopharyngeus, passes ventrally, medial to the ceratohyale and constitutes the r.descendens IX; it passes ventrally together with a nerve which branches from the r.lingualis (Fig. 67). The r.descendens possesses both visceral sensory and visceral motor fibres that innervate the mucous epithelium and muscles of the pharynx. From the r.descendens there is a connexion with the r.lingualis (Fig. 67).

Posterior to the ganglion petrosum in the 15-day stage, there is a large nerve that connects the n.vagus with the n.glossopharyngeus (Figs. 64 and 74). In the later embryos the connexion is not as clearly demonstrable as in the 15-day old embryo (Fig. 75).

Dorsal to the g.petrosum, the n.glossopharyngeus passes through the window in the posterior part of the g.cervicale supremum (Fig. 67), and together with the n.vagus, it enters the cranial cavity through the foramen jugulare, (Fig. 64). The foramen jugulare is single in most of the embryos studied, though FRANK (1954) found in his 34-day stage, separate foramina for IX and X on one side of the head. In the 36 adult heads studied, only one had separate foramina for IX and X, and then only on one side. BROCK (1937) considers this as unusual, as in other brids the metotic cartilage separates the foramina for IX and X. She moreover, maintains that the condition in *Struthio* must be considered as "incipient." (Sic!)

The n.glossopharyngeus and the n.vagus in the 27-day stage unite on the inner side of the foramen jugulare, while in the 37-day old embryo the union takes place extracranially. The combined nerves form the g.jugulare and g. superius which arise from the lateral surface of the medulla oblongata by numerous roots.

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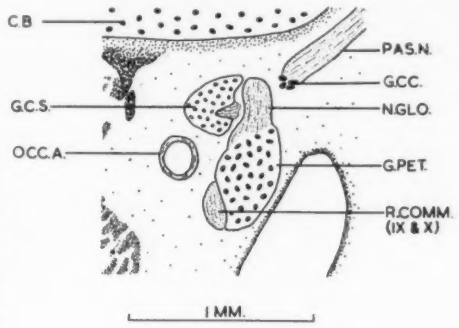

Fig. 75. Transverse section of the g.cervicale supremum and the g.petrosum in the 27-day old embryo. *C.B.*, cranial base; *G.CC.*, ganglion cells; *G.C.S.*, g.cervicale supremum; *G.PET.*, g.petrosum; *N.GLO.*, n.glossopharyngeus; *OCC.A.*, occipital artery; *PAS.N.*, parasympathetic nerve; *R.COMM. (IX* and *X)*., r.communicans between n.glossopharyngeus and n.vagus.

In *Gallus* the g.petrosum is connected to the g.cervicale supremum. The g.petrosum is also connected to the postero-dorsal parasympathetic nerve from the g.cervicale supremum as well as with the n.sphenopalatinus (v. HALLERSTEIN, 1934). These latter two connexions are not present in any stage in *Struthio*.

CORDS (1904), after v. HALLERSTEIN (1934) describes a connexion of the n.glossopharyngeus with the n.hypoglossus in *Anas*, and LUBOSCH (1932) also after v. HALLERSTEIN (1934) describes a similar connexion for birds in general. However, this connexion is not seen in any of the stages studied for *Struthio*. V. HALLERSTEIN (1934) states that the n.glossopharyngeus and n.hypoglossus may unite with each other so that the n.glossopharyngeus appears as a branch of the n.hypoglossus. In *Struthio* the n.hypoglossus crosses the r.descendens IX, but there is no exchange of fibres. V. HALLERSTEIN (1934) states that in *Gallus* there is an exchange of fibres in what he calls the glossopharyngeus crossing, "Weder die erste noch die zweite Kreuzung sind reine Ueberschneidungen". (V. HALLERSTEIN, 1934, p. 633).

D. N. VAGUS (X)

The n.vagus arises from the g.jugulare as a large nerve, passes ventrally and leaves the cranial cavity by the foramen jugulare. Immediately outside the foramen it is joined to the r.hyomandibularis by a short nerve, as previously described. In the 27-day stage where the r.communicans between the r.hyomandibularis and the n.vagus meets the n.vagus, there is a connexion with the n.hypoglossus (Fig. 76). This connexion is small (20 μ thick) and is not present in the 37-day stage. After this connexion the n.vagus passes ventrally medial to the ceratohyale and alongside the a.carotis dorsalis.

V. HALLERSTEIN (1934) describes for *Gallus* the n.vagus—n.hypoglossus crossing, calling it the vagus crossing of the n.hypoglossus. In this crossing in *Struthio*, contrary to the condition in the n.hypoglossus—n.glossopharyngeus

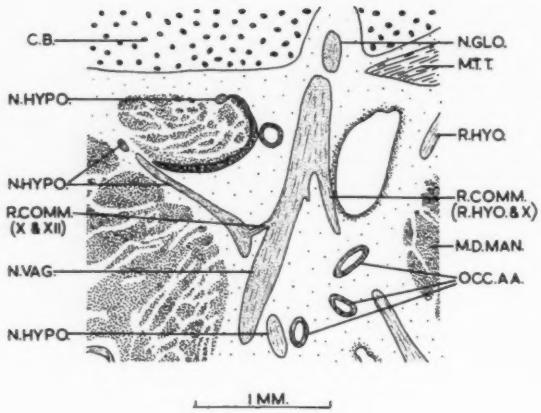

Fig. 76. Transverse section to illustrate the r.communicans between the n.vagus and n.hypoglossus in the 27-day old embryo. *C.B.*, cranial base; *M.D.MAN.*, m.depressor mandibulae; *M.T.T.* m.tensor tympani; *N.GLO.*, n. glossopharyngeus; *N.HYPO.*, n. hypoglossus; *N.VAG.*, n.vagus; *OCC.AA.*, occipital arteries; *R.COMM.* (*R.HYO.* and *X*)., r.communicans between r.hyomandibularis and n.vagus; *R.COMM.* (*X* and *XII*)., r.communicans between n.vagus and n.hypoglossus; *R.HYO.*, r.hyomandibularis.

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crossing, there is an exchange of fibres. There is no r.laryngeus in *Struthio*, and v. HALLERSTEIN (1934) states that this branch of the n.vagus in birds is lacking.

E. N. ACCESSORIUS (XI)

The n.accessorius in the normal course of events is a motor portion of the n.vagus, and in the mammals it arises from a number of roots independent of the n.vagus, emerges together with the n.glossopharyngeus and n.vagus and innervates the m.trapezius (VERSLUIJS, 1924).

Perhaps because of the inadequacy of the staining technique employed, it was impossible to demonstrate the presence of the normal type of n.accessorius passing with the n.vagus and n.glossopharyngeus. In all the embryos studied, however, a nerve which may represent a degenerate type of n.accessorius is present, leaving the g.jugulare + g.superius posteriorly. In the early stages of development the nerve is thick and extends for a considerable distance along the lateral side of the spinal cord. The older the embryo, the smaller the nerve becomes, and in the 27-day stage, the nerve, though distinct, is thin and projects slightly beyond the foramen magnum. In the 37-day stage, the nerve is considerably reduced and extends only a short distance alongside the medulla oblongata. The condition in the adult was not observed.

F. N. HYPOGLOSSUS (VII) (Fig. 67)

The n.hypoglossus aries as three independent nerves passing through their own foramina in the posterior part of the skull, and each possesses an antero-ventral and a ventral branch. The three antero-ventrals unite to form the main nerve, the n.hypoglossus (Fig. 67). The first and second branches unite first,

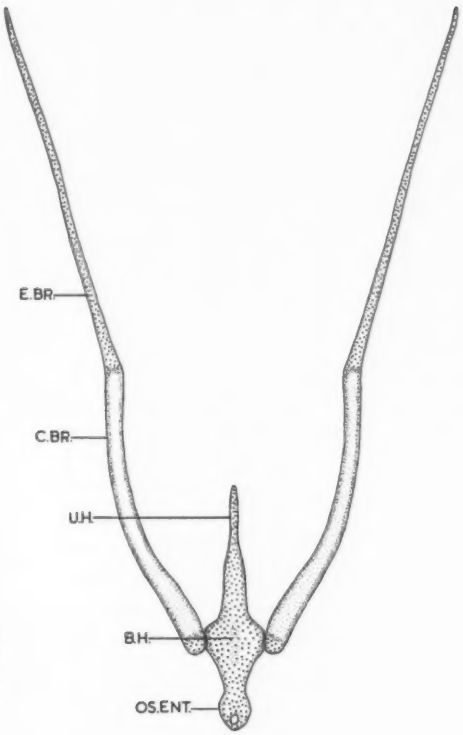

Fig. 77. Graphic reconstruction of the hyoid apparatus in the 37-day old embryo. × 3·3. Ventral aspect. *BH.*, basihyale; *CBR.*, ceratobranchial; *EBR.*, epibranchial; *OS.ENT.*, "os entoglosum"; *UH.*, urohyale.

forming a single nerve that has a connexion with the n.vagus and soon after joins with a branch from the third root (Fig. 67).

The n.hypoglossus now passes anteriorly and slightly ventrally, medial to the ceratohyale, and in the region ventral to the basihyale, the left and right hypoglossal nerves unite and give off a number of radicles which innervate the tongue muscles. Before this union takes place both hypoglossal nerves give off radicles that innervate the hyoid musculature.

The three ventral branches innervate the neck muscles.

THE HYOID APPARATUS (Fig. 77)

The hyoid apparatus of *Struthio* is for the greater part cartilaginous, unlike the usual avian condition. In the 27 and 30-day stages, the hyoid is still wholly cartilaginous and consists of three pieces: the medial basal portion, and the two lateral cornua articulating with the basal plate.

In the 37-day stage (Fig. 77) the anterior parts of the cornua have become perichondrally ossified, thus forming the ceratobranchials. The distal end of

the ceratobranchial is continued as a thin cartilaginous portion which extends to the posterior end of the skull: this represents the epibranchial. Both the cerato- and epibranchials are rod-like and circular in cross-section.

Proximally the ceratobranchial is tipped by cartilage and is movably articulated to the median cartilaginous plate; it is held in position by dense connective tissue.

The median basal plate is wholly cartilaginous and is in no way sub-divided either by ossification or by being laid down as separate areas of chondrification. It is dorso-ventrally flattened, and between the articulatory facets for the ceratobranchials, it is broad, and would therefore topographically constitute the basihyale. Anteriorly the basihyale narrows slightly, and broadens again into a disc-like structure in which is situated a foramen. This anterior portion is situated in the tongue and therefore corresponds to the "os entoglossum".

Posteriorly the basihyale narrows and forms a rodlike structure that is broad at its base and narrows distally to a point. This portion of the basal plate therefore represents the urohyale, though it is in no way separated from the basihyale.

In the adult the same condition holds as in the 37-day stage, with only the ceratohyale ossified, all other portions remaining cartilaginous.

In birds, the ossification of the hyoid, other than the epibranchials, is possibly due to feeding habits (MOLLER, 1930). In *Struthio* the grazing habit makes the use of the tongue necessary in feeding, and this would then account for the persistent cartilage. The persistent cartilage could also be considered as a neotenic character, seeing that in the skull of *Struthio* there are numerous other neotenic features.

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(a) Hyoid Musculature (Fig. 78)

Like the trigeminus musculature, the hyoid musculature has undergone reduction, or it has not reached the same degree of complexity as is has in the flying birds. This simplification of the hyoid musculature could possibly be regarded as a modification due to the grazing feeding habit of *Struthio* as compared with the specialized feeding habits of a large number of flying birds, especially the "honeysuckers" (*Blütenbesuchender*), as described by MOLLER (1930).

The space between the two halves of the lower jaw is filled anteriorly by a muscle plate in which the fibres meet in the mid-ventral line by means of an aponeurosis, which in *Struthio,* is very narrow and not distinct. Its origin is on the dorso-medial surface of the two halves of the lower jaw. This muscle is innervated by a branch of the r.mandibularis externus, and therefore is a derivative of the constrictor I ventralis: the m.intermandibularis.

MOLLER (1930) describes in *Sericotes, Cinnyris* and *Anthornis* a muscle

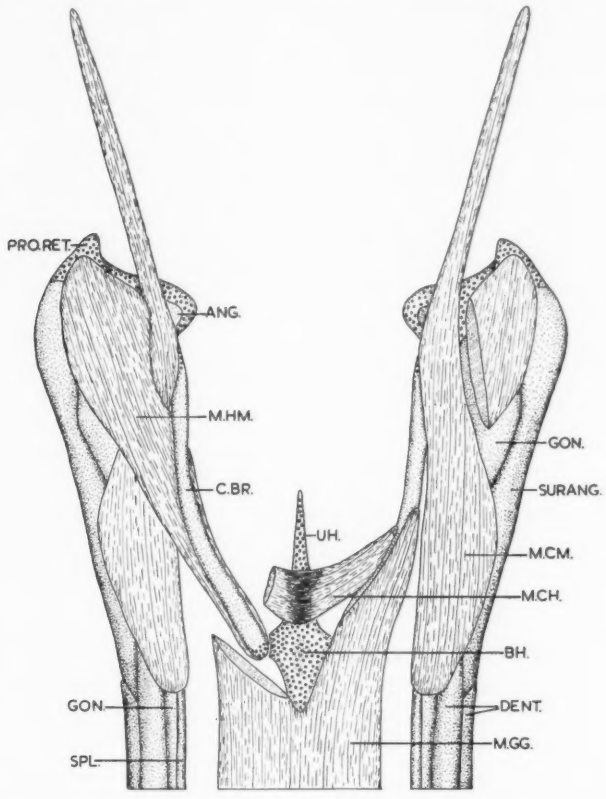

Fig. 78. Graphic reconstruction of the hyoid musculature in the 37-day old embryo. × 3·3. Ventral aspect. *ANG.*, angular; *BH.*, basihyale; *CBR.*, ceratobranchial; *DENT.*, dentary; *GON.*, gonial; *M.CH.*, m.ceratohyoideus; *M.CM.*, m.ceratomandibularis; *M.GG.*, m.genioglossus; *M.H.M.*, m.hyomandibularis medialis; *PRO.RET.*, processus retroarticularis; *SPL.*, splenial; *SURANG.*, surangular; *UH.*, urohyale.

plate identical to that of *Struthio* topographically, and calls it the m.mylohyoideus. Since he has made no mention of this muscle, it can be assumed that what he has called the m.mylohyoideus, is, in fact, the m.intermandibularis. Weichert (1951) quite correctly states that the m.intermandibularis is derived from the constrictor ventralis I and that it gives rise to the m.mylohyoideus and a part of the m.digastricus in mammals.

Ruge (1896) gave a generalized plan of the subdivisions of the hyoid muscle plate, one of these being a m.intermandibularis which, according to Brock (1938), is a hyoid intermandibularis and must not be confused with the trigeminal intermandibularis. The hyoid intermandibularis is innervated by the facial, and as Brock (1938) correctly states, the true intermandibularis is entirely innervated by the trigeminal nerve in amniotes. In *Struthio* the m.inter-

mandibularis is innervated by the trigeminal, and it is obvious that MOLLER (1930), in calling the muscle the m.mylohyoideus, is, in fact, using the mammalian terminology.

1. *M.Ceratomandibularis* (Fig. 78) (= m.genio-hyoideus, GADOW, 1883)

This muscle in *Struthio* is large and well-developed. It arises on the medio-ventral surface of the mid-region of the lower jaw and extends posteriorly, becoming inserted onto the epibranchial which it completely encircles (Fig. 56). It receives its innervation from the n.hypoglossus.

In *Cinnyris, Anthornis* and *Sericotes* (MOLLER, 1930) the m.ceratomandibularis is divided into median and lateral portions, both of which become inserted onto the epibranchial and arise on the medial surface of the lower jaw. In *Struthio* the muscle is undivided throughout its length. This muscle is used for pulling the hyoid apparatus forwards.

2. *M.Hyomandibularis Medialis* (Fig. 78) (= m.serpihyoideus, GADOW, 1883)

The m.hyomandibularis is usually divided into a lateral and a medial portion. This is the antagonistic muscle of the m.ceratomandibularis, and pulls the hyoid apparatus back. In *Struthio* it has its origin on the postero-ventro-lateral margin of the lower jaw; the fibres pass anteriorly and slightly medially ventral to the m.ceratomandibularis, to become inserted onto the latero-dorsal side of the ceratobranchial.

In *Anthornis* the m.hyomandibularis medialis is large, arises caudo-laterally on the lower jaw, and becomes inserted onto the urohyale (MOLLER, 1930). It is absent in both *Sericotes* and *Cinnyris* (MOLLER, 1930). The m.hyomandibularis lateralis (= m.stylohyoideus, GADOW, 1883) of *Anthornis* is stated as passing parallel to the m.hyomandibularis medialis and becoming inserted onto the ceratobranchiale immediately in front of the articulation with the basihyale (MOLLER, 1930). It could be argued that the muscle in *Struthio* is the m. hyomandibularis lateralis, but it must be noted that in *Cinnyris* it arises on the posterior part of the skull and is inserted on the urohyale and ceratobranchiale, while in *Sericotes,* though having the same origin as in *Cinnyris,* the insertion is on the "os entoglossum" (MOLLER, 1930). Further, it must be borne in mind that in *Anthornis,* no mention is made of the origin of the m.hyomandibularis lateralis, but it could be assumed that it has its origin on the posterior part of the skull. If this is true then the muscle in *Struthio* must be the m.hyomandibularis medialis.

3. *M.Genioglossus* (Fig. 78)

This muscle is unpaired anteriorly, but posteriorly it is divided into two lateral portions. It arises anteriorly on the premaxillary and the fibres pass posteriorly dorsal to the m.intermandibularis. In the region of the cartilaginous

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"os entoglossum" they divide, pass slightly laterally and become inserted on the latero-ventral side of the ceratobranchiale.

The m.genioglossus is absent in *Sericotes,* but is present as paired muscles in *Cinnyris* and *Anthornis.* The origin is the same as in *Struthio,* but the insertion is not only on the ceratobranchiale but also on the "os entoglossum" and on the basihyale (MOLLER, 1930).

4. *M.Ceratohyoideus* (Fig. 78) (= m.ceratoglossus, GADOW, 1883)

This is a small muscle with fibres passing laterally from the point of origin, which is a mid-ventral raphe, ventral to the urohyale, to their point of insertion on the medial surface of the ceratobranchiale anteriorly. This muscle lies dorsal to the m.intermandibularis. In *Sericotes, Anthornis,* and *Cinnyris,* as in *Struthio,* it is small, but differs from that of *Struthio* in that it is inserted on the basihyale and "os entoglossum", but it has the same insertion, on the ceratobranchiale.

All these muscles are innervated from branches of the n.hypoglossus.

THE CRANIAL GLANDS

(a) *Nasal Glands* (*Glandes nasales*) (= supraorbital glands, MARPLES, 1932)

The nasal glands of *Struthio* are dorsally situated on the skull. In both embryo and adult, the gland is bounded medially and posteriorly by the frontal, and anteriorly by the lacrimal. In a few adults the orbital ridge of the frontal is produced into an anterior process which is syndesmotically joined to the processus supraorbitalis of the lacrimal, thus forming a foramen in which are enclosed the nasal glands. This condition is present in only a few of the adult specimens, the normal condition being one in which there is no lateral osseous border to the gland.

The gland in both embryo and adult overlies part of the frontal anteriorly, which becomes excavated for this purpose; the gland is covered anteriorly by the posteromedial border of the lacrimal.

In cross-section the gland is approximately triangular, with the base dorsally situated, and with the median surface bounded by the frontal and the lateral surface by the orbital cartilage.

In *Anas,* the nasal gland is situated on the lateral margin of the frontal, and extends from the postero-dorsal point of the lacrimal to a point posterior to the orbit (GREWE, 1951). The large size of the gland in *Anas* is a result of its aquatic habit. The gland reaches its maximum size in marine birds (PORTMANN, 1950).

The gland receives its blood supply from the supraorbital artery which is a

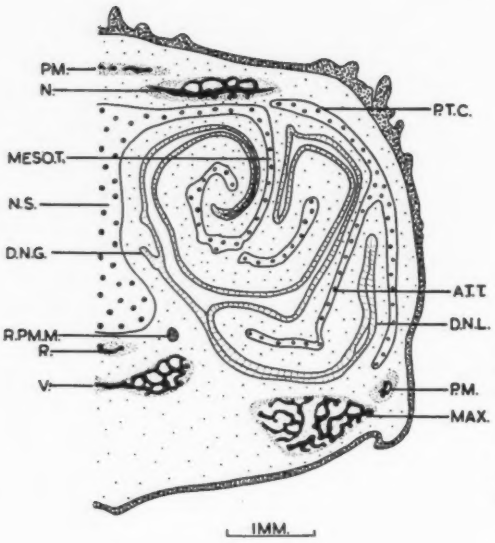

Fig. 79. Transverse section through the nasal region in the 27-day old embryo. *A.T.T.*, atrio-turbinal; *D.N.G.*, duct of nasal gland; *D.N.L.*, ductus naso-lacrimalis; *MAX.*, maxillary; *MESO.T.*, mesoturbinal; *N.*, nasal; *N.S.*, nasal septum; *P.M.*, premaxillary; *P.T.C.*, parietotectal cartilage; *R.*, rostrum; *R.P.M.M.*, r.premaxillaris medialis; *V.*, vomer.

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branch of the temporal artery, and is innervated by a branch of the parasympathetic nervous system arising from the ganglion sphenopalatinum. The blood supply and innervation of the gland in *Struthio* are totally different from that given for *Anas*, where the gland is supplied with blood from the a.ophthalmica, and is innervated by the r.lateralis nasi (GREWE, 1951, and MARPLES, 1932).

In the 27-day stage the duct, leading from the anterior end of the gland, passes ventrally, medial to the lacrimal, and then anteriorly for a short distance dorsal to the r.praemaxillaris medialis. It then turns sharply dorsally to open into the nasal sac medially (Figs. 63 and 79). There is a single duct from each gland in *Struthio*, and it therefore differs from that of *Anas*, where there is a lateral and a medial duct from each of the nasal glands (GREWE, 1951).

Owing to the thickness at which the sections were cut, it was impossible to determine accurately, the histological details of either the glands or the ducts. The lumen of the duct is, for its whole course from the gland to the nasal sac, still partly occluded. At its distal end it is completely closed, and is widest nearest the gland. The duct continues posteriorly, and breaks up into small ducteoles, which further break up into a large number of diverticulae, which differ little from the duct and are without a lumen.

In the 37-day stage, the gland has increased considerably in size, and is composed of distinct lobules, separated from each other by connective tissue. Each lobule consists of terminal portions of the diverticula, which have no lumen, and branches of the main duct, which have, in this stage, fairly large lumina (Fig. 80). Such branches of the main duct unite, giving rise to a single duct which has a very large lumen bounded by a single layer of columnar

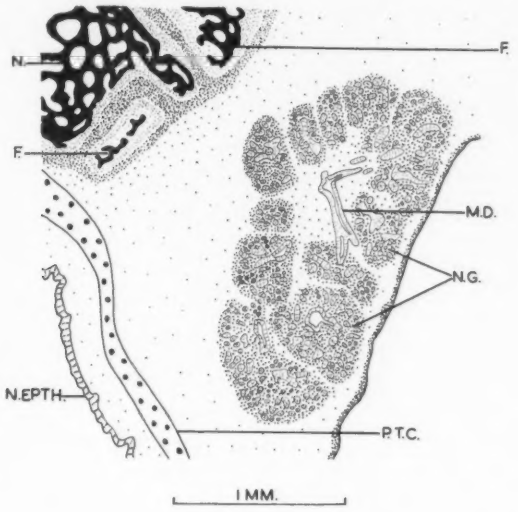

Fig. 80. Transverse section through the nasal gland of the 37-day old embryo. *F.*, frontal; *M.D.*, main duct of nasal gland; *N.*, nasal; *N.EPTH.*, nasal epithelium; *N.G.*, nasal gland; *P.T.C.*, parietotectal cartilage.

epithelial cells. The nuclei of these cells are oval, highly refractive and distally placed.

The main duct follows a course identical to that described for the 27-day stage, but during its course medial to the lacrimal, it is joined by small diverticulae identical to those of the main gland.

During ontogeny the duct arises from the medial surface of the nasal sac anteriorly, as an evagination of the nasal epithelium. The tube is short and entends only a short distance ventrally. The duct at this stage has no well-defined wall and is solid. By the 17-day stage, the duct has increased considerably in length and terminates between the eye and the septum dorsally, as three ducts, all of which are solid, as is also the main duct. The epithelium in this stage is more clearly defined, forming a distinct layer of cuboidal cells with the future lumen still absent. NITZSCH (1820) states quite correctly that the ducts develop as outgrowths of the nasal cavity, and such ducts are solid in early stages.

It is quite certain that from the formation of this gland, and the nature of the duct and the gland, it is homologous with the supraorbital gland of *Anas*, as described by GREWE (1951), even though its position in *Struthio* is not the same. This can probably be explained by the circumstance that the gland is far better developed in aquatic birds than in terrestrial birds (PORTMANN, 1950).

NITZSCH (1820) described five possible positions for the nasal glands in birds, and therefore it seems wise to drop the term "supraorbital glands" in preference to nasal glands, since these glands vary so considerably in size and in some birds are not "supraorbital" in position.

(b) *Lingual and Buccal Glands*

The tongue and posterior part of the palate are supplied with mucus-secreting glands only. The position of these glands is best observed in the 37-day old embryo in which the cells constituting the glands are filled with mucus.

In the region of the choanae, lateral to the maxillary and palatine arthrosis, the connective tissue immediately beneath the buccal epithelium is beset with small racemose mucus secreting glands, each gland possessing a large lumen. The glands are lined by a single layer of columnar epithelial cells.

Immediately posterior to the posterior flanges of the vomer, the connective tissue beneath the epithelium covering the rostrum possesses mucus secreting glands, which are larger than anywhere else in the buccal cavity. The palate has three tracts of mucus glands anteriorly, which then unite posteriorly in the buccal cavity, to form a single broad glandular area.

The tongue is also covered distally by these mucus secreting glands, which are similar to those on the palate. Posteriorly the glandular region of the tongue is confined to two lateral tracts which end at the base of the organ. Dorsally the tongue is devoid of glands, except at its very distal extremity.

Mucus glands are also present on the dorsal surface of the lower jaw immediately anterior to the point where it articulates with the quadrate. There are two tracts of glands laterally placed: they are narrow and rather short.

In the 27-day stage, the glandular tracts are identical to those of the 37-day old embryo, but the glands are still very poorly developed and show no mucus secreting cells. They are present as evaginations of the buccal epithelium, and the diverticulae of the glands are solid.

(c) *The Harderian Gland* (Fig. 63)

This is a large gland situated anteriorly in the orbit and lying ventrally between the eye and the interorbital septum. The duct opens to the exterior on the anteroventral corner of the eye beneath the membrana nictitans. It follows a course postero-medially lateral to the planum antorbitale anteriorly, and dorsal to the m.obliquus inferior posteriorly, until it divides up into the branches giving rise to the gland itself.

The gland is embedded in dense connective tissue and in the 27-day stage, the duct and its main branches have large lumina, and are lined by several layers of spherical cells. The diverticula of the ductules are solid.

In the 37-day stage, the lumen of the duct is very large and the wall very thin. It is difficult to establish the nature of the cells lining the duct because of the thickness of the sections. In this stage, the main duct leading to the gland is surrounded by diverticulae of the duct before it divides up into small ducts. These diverticulae and those that constitute the gland proper are all lined by columnar epithelial cells, as are the ductules and the mesial end of the main duct.

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The gland is innervated by a branch of the parasympathetic nervous system, arising from the g.sphenopalatinum and it receives its blood from the temporal artery.

SUMMARY

1. The adult palate of *Struthio* shows no variations, except for the vomer-pterygoid distance, which varies quite considerably.
2. During ontogeny, the palatal process of the premaxillary forms an arthrosis with the vomer. This arthrosis is lost in the adult.
3. The vomer is always forked posteriorly, and for the greater part trough-shaped and slides over the rostrum.
4. There are two synovial arthroses, one between the pterygoid and quadrate, and the other between pterygoid and processus basipterygoideus.
5. The palatal bones are firmly cemented to one another.
6. The mesethmoid appears on the dorsal surface and is surrounded by the nasals laterally, premaxillary anteriorly and frontals posteriorly.
7. Circumorbital bones are reduced to a lacrimal and a jugal.
8. There is no interparietal.
9. Nasal process of the premaxillary is unpaired.
10. Premaxillary ossifies from a single centre.
11. Parasphenoid rostrum inflated.
12. Single ossification of the interorbital septum to form the mesethmoid.
13. Orbitosphenoid absent.
14. Postorbital cartilage, pila antotica spuria and pila antotica vera ossifies as the pleurosphenoid.
15. Only prootic and opisthotic present in the auditory region.
16. Kinesis limited.
17. Absence of a fissura cranio-facialis.
18. Absence of the so-called "mesokinetic" joint.
19. The anterior cranial bones are thin and flexible particularly the nasal process of the premaxillary.
20. The quadrate has a single elongated condyle of the processus oticus, which articulates with the squamosal and prootic.
21. Meckel's cartilage excluding the pars articularis, is resorbed.
22. The dentary ossifies as a median anterior element, and the left and right hand halves are not joined in a mental symphysis.
23. The median proximal bone in the lower jaw is the gonial.
24. Five dermal bones present in the lower jaw.
25. There is one replacing bone, the articular.
26. The trigeminus musculature shows reduction.

27. The parasympathetic nervous system shows a reduction during ontogeny.
28. The cranial nerves are typical for the class *Aves*.
29. Hyoid apparatus cartilaginous except for the ceratobranchiale.
30. Hyoid musculature unspecialized.
31. *Struthio* is neotenic.

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SOME BIOCHEMICAL AND ELECTRO-CARDIOGRAPHICAL DATA ON BADGERS

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In the last few years there has been a steadily increasing interest in hypothermia because of its usefulness in certain kinds of surgery. A great number of investigations concerning the physiology of man and homoiothermic animals in the hypothermic state have recently appeared. Such investigations have also been extended to include the hibernators, i.e. animals which have the ability of decreasing their body temperature in the winter to only a few degrees above zero. Thus in our laboratory the hedgehog has been subject to study (1—5). Another animal of interest in this connection is the badger. The winter sleep (6) of this animal resembles that of the hibernators in immobility and loss of body weight, while the body temperature—on the other hand—is at a constant homoiothermic level throughout the winter (6). — It seems, however, as if very few studies have been made on the physiology and biochemistry of badgers. We had the opportunity to get some captured animals on which we carried out some biochemical and electrocardiographical studies, which are presented below.

METHODS AND MATERIAL

At the beginning we had access to four badgers (Meles meles L) which were used for studies from June 1955 to May 1956.

Blood samples were taken in June 1955 (no. 1), in August 1955 (nos. 2, 3 and 4) in October 1955 (nos. 2, 3 and 4), in February—March 1956 (nos 2, 3 and 4) and in May 1956 (nos. 3 and 4) by venous puncture after the animals had been anesthesized with ether and 2 cc nembutal (Abbott) intramuscularly. One animal (no. 1) died in June 1955 because of an overdosage of ether in connection with the taking of a blood sample. Another (no. 2) was found dead one morning in March 1956. The cause of death is unknown. These deaths are the reason why only results from two animals are given in the tables from the latter part of the investigation.

The methods used in determining hemoglobin, red and white blood corpuscles, differential count, thrombocytes, total eosinophils, reticulocytes, hematocrit, plasma proteins, protein-bound iodine, serum sodium, serum potassium and blood sugar have been reported earlier (3). Determination of serum calcium was made acc. to a method by NIELSEN (7) (somewhat modified) and of serum cholesterol acc. to a method by ABELL et al (8).

The body temperature was determined in centigrades by a thermometer introduced 7 to 8 cm into the rectum.

The animals received kitchen garbage and water all the year. One animal (no. 4) was kept in a room where the air temperature during winter was about 16° C. The others were kept outdoors (nos. 1, 2 and 3).

The two animals which remained in June 1956 were cooled in a specially built refrigeration box (9) after anesthesia with ether and nembutal. During the whole refrigeration period the animals were hyperventilated with a respirator (Lundia) at a rate of 24 respirations/min.

Electrocardiograms were obtained with an electrocardiograph (Elema Triplex). During refrigeration tracings were recorded for every two degrees decrease of the body temperature.

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RESULTS AND DISCUSSION

Data on body weight and temperature are given in table I. The animals showed an average increase in body weight of 3.1 kg from August to October but afterwards decreased again in weight. No. 2. showed only a slight decrease. This animal was found dead shortly afterwards. The other animal which was out of doors (no. 3) lost much more in weight (4 kg) than the animal (no. 4) which was kept in a warm room all the winter (1.4 kg) although both animals received as much food as they wanted. In May only the weight of one animal (no. 3) was determined. This value was a little lower than the one found in February.

Although some early investigators (10, 11, 12) considered the badger to be a hibernator, it is now generally accepted (13, 14, 15) that this is not the case. A hibernator is a mammal which during the winter can decrease its body temperature to that of the surroundings if this temperature is within the range of 0° to 15° centigrades. In this state, which is interrupted with rather long intervals, the animals are almost motionless, the heart rate is very slow but fairly regular and the metabolic rate reflected in the oxygen consumption is very much decreased. — As the body temperature of the badgers in the winter never decreased to that of the surroundings (table I) and the animals were found walking about quite often, it is obvious that the captured badger is not hibernating.

HEMATOLOGICAL RESULTS

Animals nos. 3 and 4 showed a slight decrease of the hemoglobin value in October while the red corpuscle value decreased in no. 3 and was mainly unchanged in no. 4. When the winter was over the hemoglobin values increased; in no. 4 this increase appeared already at the end of February. At his time there was also a great increase in the number of red corpuscles. Animal no. 2 differed from the others (table I). The low values in March can with great probability be looked upon as a symptom of the disease from which it died shortly afterwards.

The data on the number of white blood corpuscles (table I) did not show any consistent changes. Thus, unlike in the hedgehog, no decrease was seen in the winter. The differential count (table I) was mainly unchanged although there was a tendency of the neutrophils to be slightly increased in October and May at the expense of the lymphocytes.

The number of total eosinophils and thrombocytes (table I) showed great variations but no consistent change could be found. The values were lower than those seen in dogs (16, 17) and non-hibernating hedgehogs (3). — No consistent changes were found in the reticulocytes either (table I). The high value seen in animal no. 2 was probably secondary to the anemia.

Hedgehogs in hibernation showed an increase of the hematocrit in January. In March this increase was followed by a decrease. In one badger (no. 4) there was an increase in February but in the other (no. 3) no change was found (table II).

The serum sodium concentration remained mainly unchanged during the different seasons in accordance with the results found in hedgehogs (3). The serum calcium concentration remained unchanged in no. 4 and showed an increase in August in nos. 2 and 3. The serum potassium concentration (table II), in October, showed a decrease, which persisted in February—March but in May it was restored to the level found in August. This resembles Suomalainen's findings in hedgehogs which we, however, have not been able to verify.

Typical of most hibernators is a decrease in blood sugar during hibernation (3). In the badgers the values were rather a little higher in February—March than in other seasons (table II).

The protein-bound iodine values (table II) were of the same order as those found in other animals. They did not show any consistent changes in different seasons. This is in contrast with hedgehogs which show a decrease during hibernation (3).

The electrophoreses (table II) of the badgers' plasma proteins in most cases differed from those of certain other animals like the hedgehog and the dog, having only five distinct fractions with the method used. Of course it is open for

TABLE

Date	Animal no.	Weight in kg	Rectal temp. in °C	Hemoglobin in per cent	Red corpuscles (mill./mm³)	White corpuscles (number/mm³)
18/8—55	2	12.0		93	7.3	7.200
25/8—55	3	13.5	38.0	98	9.1	4.000
29/8—55	4	13.5	37.4	96	7.5	4.900
24/10—55	2	14.5	35.5	93	7.7	3.900
26/10—55	3	16.5	36.5	84	7.5	5.900
20/10—55	4	17.4	36.3	86	7.7	9.500
5/3—56	2	14.0	37.0	50	4.0	5.700
1/3—56	3	12.5	37.0	84	11.3	6.600
23/2—56	4	16.0	36.0	104	11.9	4.500
22/6—55	1			71	6.0	7.400
28/5—56	4		36.0	102	8.6	4.500
31/5—56	3	12.0	37.0	97	8.4	4.000
28/5—56	4		18.0	104	7.5	1.300
31/5—56	3		14.0	94	9.0	700

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TABLE

Date	Animal no.	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Hematocrit (in per cent)	Protein-bound idodine (in γ per 100 cc)	Blood sugar (in g per 100 cc)
18/8—55	2	148	3.2	4.8	44.4		
25/8—55	3	129	4.2	5.3	40.6	3.1	0.106
29/8—55	4	138	4.0	5.0	35.0		0.125
24/10—55	2	150	2.5	4.0	43.4	4.3	0.135
26/10—55	3	154	4.0	4.6	37.0		0.072
20/10—55	4	144	3.2	4.9	39.8	3.6	0.262
5/3—56	2			4.7	28.0	5.2	0.184
1/3—56	3	149	3.7	4.0	36.8	2.7	0.152
23/2—56	4	150	3.5	4.8	49.2	3.4	0.174
22/6—55	1	150	3.8	5.1	28.0	3.1	
28/5—56	4	154	4.4	4.8	45.5	3.3	0.100
31/5—56	3	147	4.3	4.3	44.1	3.8	0.090
28/5—56	4	154	5.4	5.0	50.7	2.9	0.080
31/5—56	3	139	4.3	3.9	41.8	4.0	0.110

I

Differential count (per 100 counted cells)					Total eosinophils (number/mm³)	Thrombocytes (number/mm³)	Reticulocytes (per 1000 cells)
Neutrophils (in per cent)	Eosinophils (in per cent)	Basophils (in per cent)	Lymphocytes (in per cent)	Monocytes (in per cent)			
54	1	—	42	3	160	272.000	0
59	—	—	38	3	16	268.000	1
53	1	—	44	2	170	750.000	15
70	—	—	30	—	16	270.000	6
62	—	—	36	2	102	478.000	5
60	—	—	39	1	112	487.000	44
57	—	—	40	3	26	548.000	246
60	1	—	37	2	19	421.000	16
56	—	—	43	1	12	318.000	7
67	—	—	31	2	16	522.000	53
76	1	—	21	2	200	182.000	2
60	—	—	39	1	12	402.000	0
57	—	—	38	5	2	1.800.000	0
68	—	—	30	2	4	488.000	2

II

Total protein (in g per 100 cc)	Albumin (in g per 100 cc)	α-glob. (in g per 100 cc)	β_1-glob. (in g per 100 cc)	β_2-glob. (in g per 100 cc)	γ-glob. (in g per 100 cc)	Cholesterol (in mg per 100 cc)
6.5	3.67	0.84	0.33	0.85	0.81	
6.0	3.70	0.42	0.38	0.78	0.72	
7.1	4.27	0.50	0.35	1.11	0.87	
6.7	3.96	0.65	0.32	0.84	0.93	326
6.7	3.63	0.69	0.45	0.92	1.01	431
6.4	3.78	0.50	0.40	0.95	0.77	399
7.7	4.63	0.56	0.46	1.05	1.00	274
6.5	4.30	0.27	0.20	0.87	0.86	185
7.6	4.51	0.51	0.15	1.17	1.26	132
7.6	4.30	0.55	0.28	1.22	1.25	143
6.7	3.80	0.32	0.60	0.93	1.05	162
7.6	4.34	0.53	0.37	1.09	1.27	123
6.3	3.58	0.58	0.23	1.00	0.96	153

discussion what to call these fractions but a comparison indicates that it is an α-globulin fraction that is lacking. We have therefore decided to call the different fractions as follows: the albumin-, α-, β_1-, β_2- and γ-fraction. — The total protein and the albumin values were slightly higher in February—March, thus in accordance with values found in hedgehogs (3). This increase persisted in May as regards the total protein values. The β- and γ-fractions did not show any consistent changes although the individual alterations were rather large. In this respect, then, the badger differs from true hibernators like the hedgehog which show a decrease in the β- and γ-fractions during hibernation.

The observation has been made that the concentration of plasma cholesterol is higher in man than in other mammalian species (18); this has been offered as an evidence for the etiologic role of the blood lipids in the pathogenesis of atherosclerosis. Recently, however, BRAGDON (19) has made serum cholesterol determinations in a hibernator, Citellus columbianus, and has found comparatively high values. WILBER and MUSACCHIA who analyzed Alaskan ground squirrels (Citellus barrowensis) found a total cholesterol value of about the same order as that found in human beings (20). They were not able to find any seasonal variations, SUOMALAINEN (6) reported a high total cholesterol content (455 mg per 100 cc) in the serum of hibernating hedgehogs, while the value was much less in early summer (250 mg per 100 cc). — In table II it can be seen that at least in October the values found in badgers were high, averaging 385 mg per 100 cc. In May the average value found was 153 mg per 100 cc.

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ELECTROCARDIOGRAPHIC RESULTS

The heart rate was rather varying in different animals in different seasons. These changes were probably caused by a different anesthetic depth, although we tried to keep it as constant as possible. No consistent differences between the seasons were observed and the mean of all the determinations was 179 beats/min. It is thus of about the same size as that found in hedgehogs—181 beats/min. (5)—with a skin temperature of 32.5° C, and a little higher than the value found in dogs—146 beats/min.—with a body temperature of about 38° C and a body weight of 20—35 kg (1). The slight difference in heart rate between the hedgehogs and the badgers was astonishing as the body weights differ much (0.5—1.0 kg and 12—17 kg respectively). The slightly lower body temperature in the hedgehogs is probably not the only reason, but also a superficial anesthetic level in the badgers during the ECG recording may have played a rôle. The P-R, QRS, and Q-T intervals averaged 0.084, 0.055, and 0,201 secs., respectively. Fig. 1 a shows a typical ECG.

EFFECTS OF INDUCED HYPOTHERMIA

Two animals (nos. 3 and 4) were cooled by surface cooling in May 1956 in order to find out the animals' reaction to cold. A true hibernator is able to

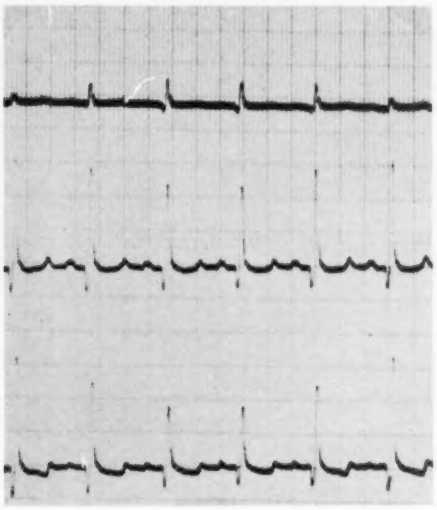

Fig. 1 a

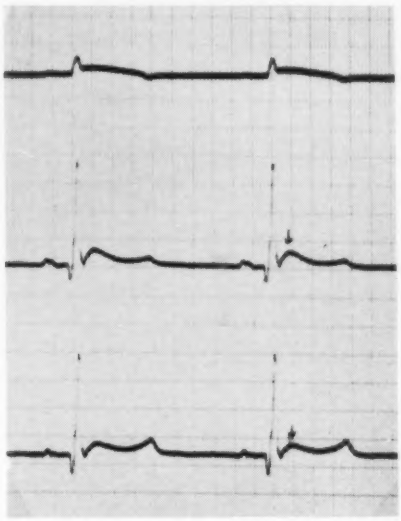

Fig. 1 b

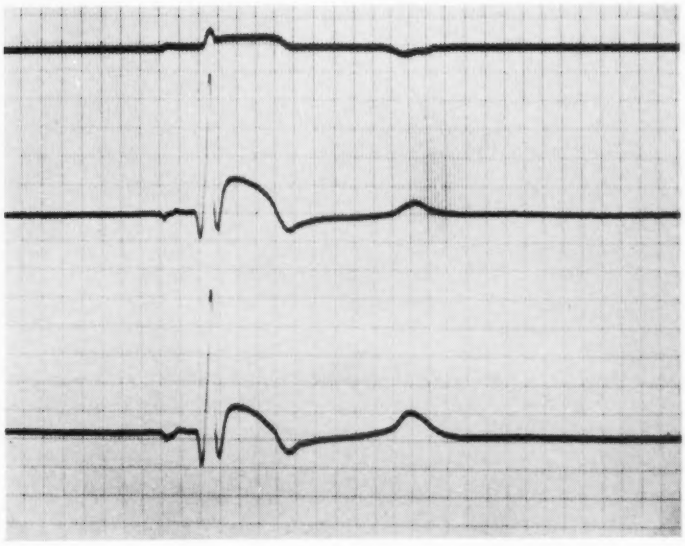

Fig. 1 c

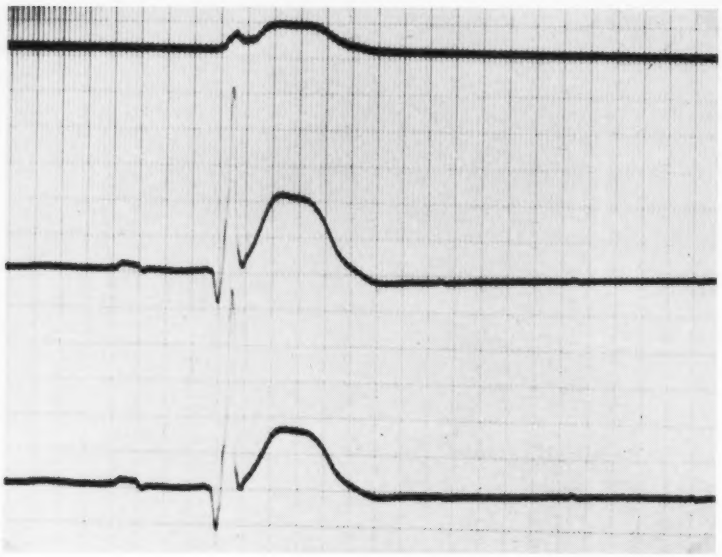

Fig. 1 d

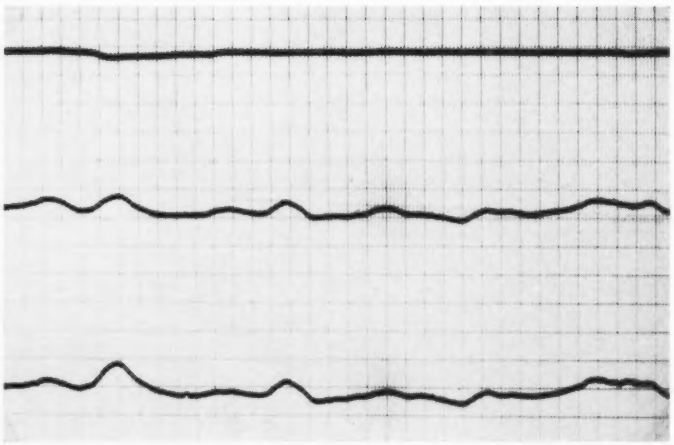

Fig. 1 e

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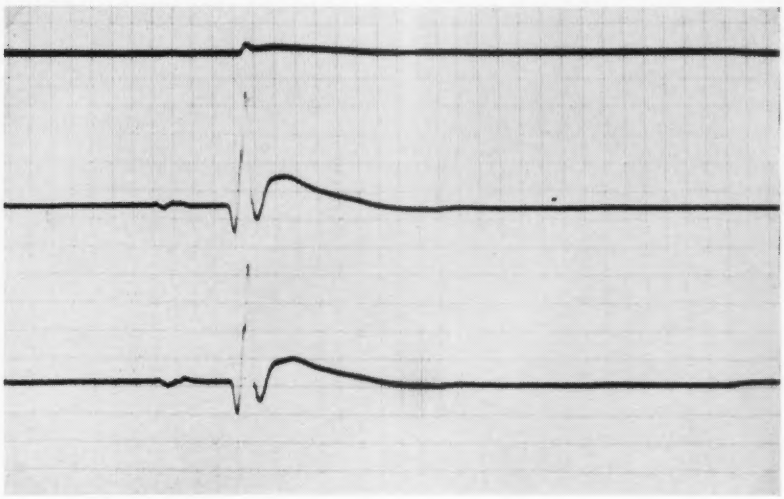

Fig. 1 f

Fig. 1. ECGs from a badger at different temperatures.

a) before refrigeration. Body temperature 37° C.
b) 26.5° C. Note the Osborn wave (↓).
c) 19.5° C. Large diphasic Osborn wave. T wave positive in leads 2 and 3.
d) 19.0° C. Large Osborn wave. No T wave is seen.
e) 15.5° C. Ventricular fibrillation.
f) 14.5° C. The ventricular fibrillation has disappeared spontaneously.

The figure shows the three standard leads. The distance between two heavy lines is 0.1 sec.

tolerate a decrease of the body temperature to just above zero with the heart still beating spontaneously, while the heart of a non-hibernating mammal stops beating at about 13° C (21). One badger (no. 4) was cooled to 19° C; at this temperature the heart rate was 43 beats/min. (at 22° C it was 21 beats/min.). After this the animal was rewarmed but died soon after the beginning of this procedure. The other animal when being cooled to 19.5° C showed a heart rate of 13 beats/min. and the rhythm was a nodal one. At 17° C the heart rate had increased to 32 beats/min., total block was persisting and auricular and ventricular premature beats appeared rather frequently. A few minutes later ventricular fibrillation was registered, which, however, had disappeared spontaneously at 14.5° C when the heart rate was 12 beats/min. (fig. 1). At 14° C no deflections were registered electrocardiographically. When after opening of the thorax the immobile heart was touched with the finger or pinched with a forceps a slow contraction appeared. In Fig. 2 the heart rate, P-R, QRS, and Q-T durations have been compared with corresponding values in dogs and hedgehogs. — An interesting finding was the very large Osborn wave (1); at the lower temperatures this was dominating. The T wave persisted to 19.5° C

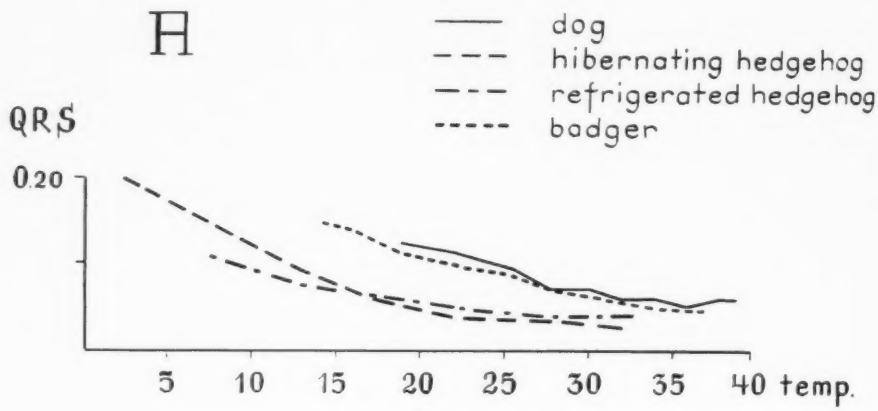

Fig. 2 A

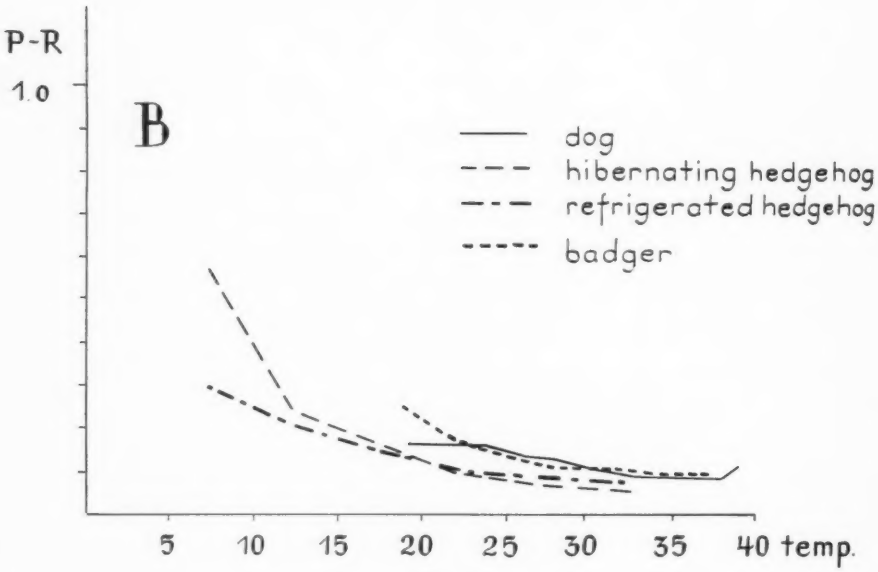

Fig. 2 B

and showed an increasing positivity. Below 19.5° C the T wave disappeared and only the large Osborn wave was seen (Fig. 1).

In tables I and II the hematologic findings at the lowest body temperature are given. Some consistent changes were observed: the number of leucocytes decreased, a fact observed both in hibernating hedgehogs (3) and cooled dogs (16). Furthermore the eosinophils decreased and the thrombocytes increased; the same changes have also been observed in hedgehogs during hibernation (3).

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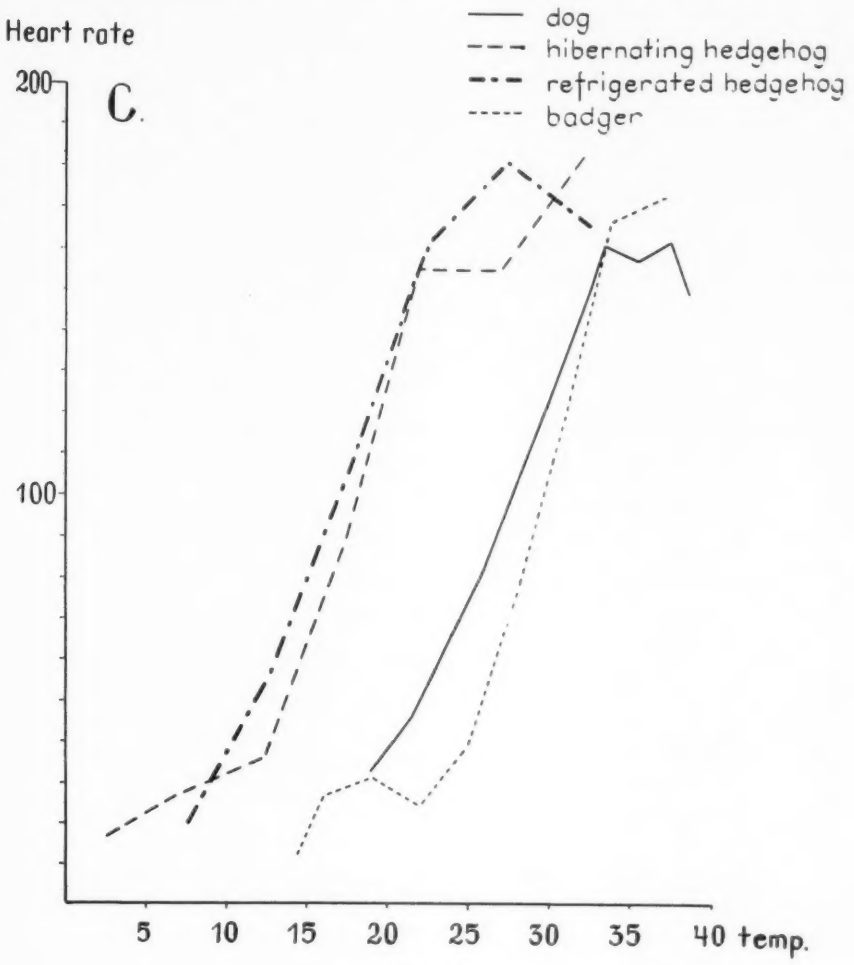

Fig. 2 C

COMPARISON WITH DATA FROM HEDGEHOGS AND DOGS

In this connection it may be of interest to compare the hematologic and electrocardiographic changes found in a hibernator when cooled with those found in the badger. Five hedgehogs (Erinaceus europaeus) were given 15—25 mg chlorpromazine (hibernal, Leo) intraperitoneally and were put into a refrigeration box (9) where cold air (0° C) was circulating. Electrocardiograms were registered with needle electrodes (see (1) for details) at different temperatures and some hematologic determinations were made before and after the refrigeration. The cooling was stopped at a skin abdominal temperature of +7° C. — More pronounced and consistent changes were found in the hemoglobin and red corpuscle values in hedgehogs than in badgers. Thus in the former there was an average decrease from 97 per cent to 81 per cent hemoglobin and from 10.1 millions/mm^3

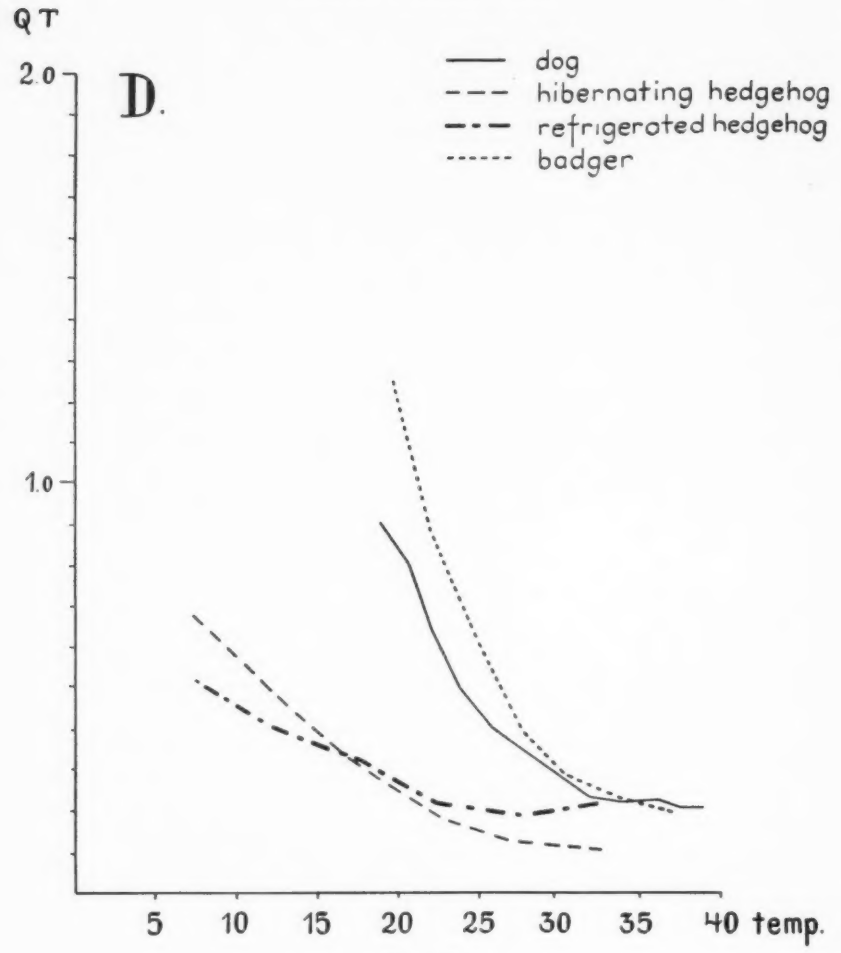

Fig. 2 D

Fig. 2. Heart rate (fig. C), P-R (fig. B), QRS (fig. A), and Q-T interval (fig. D) from dog (————), badger (· · · · ·), hedgehog arising from hibernation (— — — —) and hedgehog rendered hypothermic (— · — · — · — ·).

Abscissa: temperature in centigrades.
Ordinata: heart rate in beats/min., P-R, QRS, and Q-T in secs.

to 8.4 millions/mm³, respectively. As in the badgers there was a great decrease in the number of white corpuscles and total eosinophils from 10360 to 4140/mm³ and from 192 to 51/mm³, respectively. — The heart rate, P-R, QRS, and Q-T durations are given in Fig. 2. It is seen that the values from the badger and the dog do not differ much. The values from the refrigerated hedgehogs and those from the hedgehogs arisen from hibernation are also similar, but different from the dog and badger values. This may at least in part be explained by the difference in body weight, the weight for the badgers (12—17 kg)

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and the dogs (20—35 kg) being rather similar and greater than that of hedgehogs (0.5—1 kg). — As for the hedgehogs differences are seen in the heart rates at 27.5° C (a higher value in the refrigerated animals) and in the Q-T intervals. Below 15°—20° C the Q-T is shorter in the refrigerated animals than in the hibernating ones. — When comparing ECGs from arctic ground squirrels, NARDONE (22) found a "left bundle branch block" in the hibernating specimens and a "right bundle branch block" in hypothermic specimens. No such changes could be discovered in the hedgehogs.

CONCLUSIONS

In conclusion it can be stated that some of the recorded data indicate conditions in our badgers similar to those of a true hibernator. Thus during winter the animals are rather motionless, the body weight shows a fairly large decrease and the albumin fraction of the electrophoresis shows an increase. There are differences too, however, and these are more conspicuous. Firstly, the body temperature does not decrease and it is not probable that this is caused by the captured state since captured hedgehogs hibernate. Secondly, most of the changes in the hematologic values do not agree with those found in hedgehogs. The lack of decrease in white blood corpuscles, and blood sugar is particularly worth mentioning. Thirdly, when cooled, the badger's heart stops beating at about 14° C which is typical of non-hibernating mammals.

SUMMARY

Biochemical and electrocardiographical data have been collected in four badgers (Meles meles) in different seasons and have been compared with values from other animals.

The body weight decreased during the winter. The hemoglobin and red corpuscle values increased in winter or spring. The serum potassium values were slightly lower in October and persisted so to February-March. The total protein and albumin fraction was higher in February-March as was the blood sugar in two of the three recorded animals. Very high cholesterol values were found in October. No consistent changes were found in the other hematologic data (tables I and II).

Electrocardiograms were alike in different seasons; the heart rate averaged 179 beats/min., P-R 0.084 secs., QRS 0.055 secs., and Q-T 0.201 secs.

Refrigeration revealed interesting electrocardiographic changes: a large Osborn wave; a T wave which increased in positivity to 19.5° and afterwards became isoelectric.

It is concluded that in some aspects badgers are similar to hibernators but that most findings agree with those found in non-hibernators.

The author is greatly indebted to Drs G. Biörck and Ivar Borg for providing the animals. Thanks are due also to Mr. R. Prahl for technical assistance, to the head and personal of the Chemical and Medical Laboratory, to Dr. B. Skanse and Mr. I. Hedenskog for their help with the chemical analyses, and to Dr. W. von Studnitz for help with the electrophoreses.

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FURTHER STUDIES ON THE FERTILIZATION OF JELLY-FREE SEA URCHIN EGGS

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It was previously demonstrated that the removal of the jelly coat, which surrounds the sea urchin egg, results in an increased fertilizability of the egg (1, 2, 6). It was also observed that the jelly coat constitutes a block to heterologous fertilization and to polyspermy (2, 3, 4). These results are at variance with the "fertilizin theory" (7, 11), which claims that the presence of the intact jelly coat (= fertilizin, 11, cf. 13) favours fertilization and that the removal of the jelly causes a more or less complete loss of the fertilizability of the egg (10, 11). The depressed fertilizability of jelly-free eggs was, however, found to be dependent upon the high concentration of acid (pH 3.5—4.5) used to remove the jellies and it could not be ascribed to the loss of the jelly coats (1, 2). The injurious effect of too acid sea water was already observed by Vasseur, who found that eggs subjected to sea water of pH $\leqq 5$ showed an marked tendency to cytolysis (12). Though it did not seem very likely that acid sea water of pH 5.6—5.8 (cf. 1) would *per se* exert a favourable action on fertilization another rapid method for the removal of the jelly coats from the eggs was sought.

Lindahl and Lundin (8) described an experimental procedure for removal of the fertilization membranes from large quantities of fertilized sea urchin eggs and this method was later used with success by Lindahl et al. (9) for removing the jelly coats from the eggs of *Paracentrotus lividus*. On the basis of Lindahl's and Lundin's method one of the present writers (B. M.) has worked out a method by means of which it is possible to remove the jelly coats mechanically without producing any injury to the eggs.

MATERIAL AND METHODS

The present work was carried out at "Stazione Zoologica", Naples, with *Paracentrotus lividus* and *Psammechinus microtuberculatus* as material. In

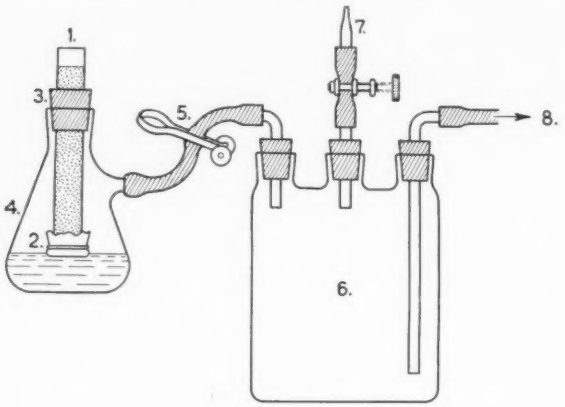

Fig. 1

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every experiment eggs from only one female were used. The egg suspension was divided into three lots; one lot was used with intact jelly coats (control), another lot was treated with acid sea water of pH 5.6—5.8 (cf. 1) to remove the jellies and the third lot of eggs was deprived of their jelly coats mechanically (see below). The three batches of eggs were simultaneously tested in fertilization rate experiments (5) in which insemination was made with the same sperm suspension.

The apparatus used to remove the jelly coats is shown in figure 1. A tube (1) is inserted airtight (3) into a 250 ml, suction flask (4) containing a small amount of sea water. The lower opening of the tube is covered with bolting silk (2) and is kept just beneath the liquid surface. The suction flask is connected through a Woolf flask (6) with a water pump (8). The jam (5) is at first kept closed which makes it possible to fill up the tube (1) with egg suspension. The negative pressure is adjusted with a jam and a capillary tube (7) to give a sucking force as weak as possible to effect a complete removal of the jelly coats. As a rule the egg suspension was passed two times through the tube.

To remove the jellies from *Paracentrotus* eggs bolting silk no. 16 xx was used wheares bolting silk no. 14 xx was found to be more suitable for removing the jellies from the somewhat bigger *Psammechinus* eggs.

The jelly-free eggs treated mechanically or with acid sea water were washed in filtered sea water before the experiments. The control eggs were subjected to the same amount of washing as were the jelly-free eggs and therefore the batch of control eggs differed from the two other batches only as regards the jelly coats. The treated eggs were stained with basic stains (e.g. toluidine blue, Janus green B) and examined in the microscope in order to make sure that the eggs were jelly-free.

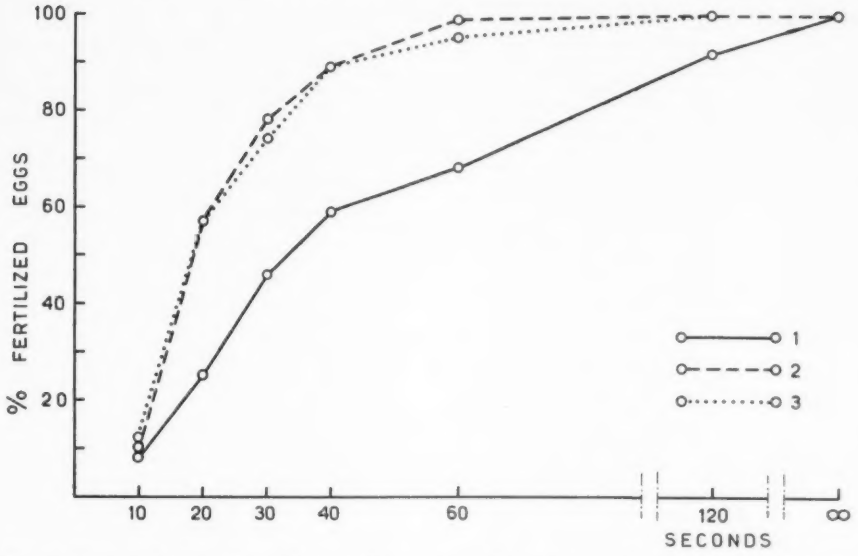

Fig. 2. Eggs and sperm from *Psammechinus microtuberculatus*. 1. Control. Eggs with intact jelly coats. 2. Eggs deprived of their jelly coats by treatment with acid sea water at pH 5.8. 3. Eggs deprived mechanically of their jelly coats. The sperm was killed 10, 20, 30 etc. seconds after insemination by adding sodium laurylsulphate to a final concentration of 0.001 per cent. Each point of the curves represents about 250 counted eggs.

RESULTS

The results revealed that eggs deprived of their jelly coats mechanically show an increased capacity for fertilization as measured with the fertilization rate method. It was also found that membrane elevation and ensuing development were quite normal after mechanical removal of the jellies. Moreover, these eggs showed almost the same fertilization rate as did eggs, from the same female, which had been treated with acid sea water at pH 5.8 (cf. figure 2).

The mechanical method of removing the jelly coats has, however, certain limitations. Thus it was observed that eggs with very compact jelly layers passed unchanged through the bolting silk and at the end of the spawning season even the most gentle negative pressure induced cytolysis in some batches of eggs. Both these types of eggs were readily deprived of their jellies by treatment with acid sea water, which latter treatment was found not to produce any damage to the eggs. The fertilization rate showed also the normal increase upon removal of the jelly coats. If only a small amount of jelly-free eggs is required and if it is possible to use eggs from only one female the mechanical

method works in most cases very well. Treatment with acid sea water is, on the other hand, the method preferred when large quantities of jelly-free eggs are needed.

DISCUSSION

The results reported above are in support of the opinion expressed by Vasseur (13, p. 25) viz. "the jelly coat is not bound to the egg surface but is completely free from it". The eggs of *Psammechinus microtuberculatus* have often a narrow, 3—5 μ wide space between the egg surface and the inner surface of the jelly coat. Vasseur's finding that protozoa may be observed between the outside of the egg and the inside of the jelly (13) has also been confirmed by the present writers.

When eggs are passed through bolting silk, segments of the jelly coat are cut off and the eggs are squeezed out of the jellies. By staining the empty isolated jelly layer with toluidine blue or Nileblue sulphate it was seen that the inner and the outer surface of the jelly coat showed the same staining properties; the inner surface appeared as distinct as did the outer. All these facts indicate that the jelly coat is an envelope loosely united to the egg. This is also suggested by the extreme ease at which the jellies are instantaneously removed from the eggs upon treatment with acid sea water. It is also evident that the improvement in the fertilization rate observed when the jelly coats are removed at pH 5.8 (1) must be ascribed to the loss of the inhibiting jelly and not to any action whatsoever of the acid sea water on the egg proper; jelly-free eggs obtained according to the two different methods used in this investigation show almost identical fertilization rates (Fig. 2).

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SEXUAL DIFFERENCES OF CAROTENOID CONTENT IN THE SKIN OF LABRUS MIXTUS KROEYER

BY

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During the last two decades skin carotenoids from a number of fish species have been extracted and examined. LÖNNBERG (1929—1940), SUMNER and FOX (1933—1935), FOX (1936) and v. EULER and HELLSTRÖM (1934) were the first authors to draw attention to this matter and the physiological problems connected with it. Lately appeared important papers by STEWEN (1948—1950) and by KRITZLER, FOX, HUBBS, and CRANE (1950). Detailed information concerning the carotenoids in general and in fishes in particular can be found in monographs and reviews by ZECHMEISTER (1934), LEDERER (1938), KARRER and JUCKER (1948), and GOODWIN (1950, 1951, 1952).

LÖNNBERG (1929) observed that alcoholic and petroleum ether extracts from the skin of *Labrus mixtus (ossifagus)* submitted to capillarity test produced on filter paper two separate pigment bands, one of red, the other of yellow colour. LÖNNBERG concluded that the skin of the fish must contain at least two different carotenoids. Later (1939) the same author established the presence of both taraxanthin and xanthophyll, and of an astaxanthin-like pigment in the skin of various specimens of *Labrus mixtus*. As far as we know, these are the only existing references to the skin carotenoids in *Labrus mixtus*.

Different genetic and external factors determine the species- and sex-specific coloration pattern of a fish. Many fishes of the family *Labridae* are known for their sexual differences in the body coloration. In *Labrus mixtus* these differences are particularly striking. The males are dark-greenish dorsally, and have a characteristic design of curved blue lines on the head, and five wide blue bands along the sides. The ventral part of the body is of bright orange-yellow colour with irregular blue spots cranially. Also the fins are orange-yellow with typically arranged blue spots. The female is brick-red all over the body. The blue design on the head is feebler and less coherent. The fins are brick-red with predominantly black spots and only a few feeble blue ones. The blue coloration depends on the presence of a complex carotenoid-proteid, the red and the yellow colour on the presence of carotenoids. Concerning the taxonomy and ecology of Labrus mixtus see CUVIER-VALENCIENNES (1839), LILJEBORG (1891), WRIGHT and SMITH (1892) and GOURRET (1901).

The aim of this investigation was to determine whether or not in Labrus mixtus there exists any sexual difference in the composition and the amount of skin carotenoids.

MATERIAL AND METHODS

About 40 specimens of both sexes have been examined during and shortly after spawning. In 18 of the specimens the content of skin carotenoids has been determined quantitatively. The fishes came from the shores of Flatholmarne at the mouth of the Gullmarsfjord on the Swedish west coast. To the technique of skin preparation and extraction, described in a previous paper (ABOLINŠ and ABOLINŠ-KROGIS, 1957), the following can be added, The very cautiously detached skin and fins must be collected in closed vessels for weighing under maintained wet-weight conditions. The skin, particularly that of the head and the fins must be minced and finely ground, as otherwise the following extraction will not be complete.

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Various analytical methods introduced by KARRER, LOEWE, and HUBNER (1935), KUHN and BROCKMANN (1932), KUHN and LEDERER (1931, 1933), KUHN, STENE and SÖRENSEN (1939), KUHN, WINTERSTEIN and LEDERER (1931), and STRAIN (1938, 1942) have been applied with some modifications.

Every extract of each single specimen has been analysed separately. Some samples (total skin extracts) were submitted to the partition with 90 % methanol. They showed to be practically completely epiphasic. Only occasionally feeble traces of pigment could be observed in the methanolic hypophase. For this reason most of the carotenoid total extracts have been submitted to further analysis without previous partition between methanol and petroleum ether.

Two different techniques of carotenoid isolation have been tried. While starting the analyses we attempted to separate the individual pigments from the total extract by using only fractioned extraction. The entire petroleum ether extract of a specimen was saponified for 3 hrs at 40° C (or for 12—24 hrs at room temperature) with 5 % KOH in 96 % ethanol. If after addition of 90 % methanol some of the pigment still remained in the epiphase, the saponification was repeated with the remainder. For the extraction of the saponified carotenoids petroleum ether (60°—70°) and distilled water were gradually added to the soapy methanol in a separation funnel. Every extracted portion of the pigment was then isolated separately. In order to transfer as much of the pigment as possible into petroleum ether the methanol was gradually acidified by adding acetic acid during the last steps of partition.

In most cases the skin carotenoid total extracts were analysed by the chromatography on the column. As the fractionated extraction had shown the complete transfer of all the saponified pigment from the soapy methanol into

petroleum ether to be rather difficult, the first chromatographic procedure was performed prior to the saponification. The adsorbent used was sucrose (prepared freshly from a crystalline product) or talc. Repeated filtration through sucrose followed by development of the column with excess of light petroleum proved to be an appropriate and convenient procedure for the separation of the two main components of fish skin carotenoid extracts. However, during warm wet summer days and at the sea coast with permanent high humidity of the air sucrose had to be replaced by a somewhat stronger adsorbent like powdered talc. In some cases, instead of repeated chromatography on sucrose (talc), the chromatography on sucrose (talc) followed by chromatography on precipitated calcium (zinc) carbonate was used for the purification of the percolate from the first sucrose column.

The adsorbates were eluted from the columns with light petroleum with 0.5 to 1.0 % ethanol. After the usual washings and dryings both eluates and percolates were saponified as described earlier. The hypophasic saponified carotenoids were then transferred into petroleum ether, and again washed and dried. The subsequent chromatography of saponified eluates as well as saponified percolates was carried out on calcium (zinc) carbonate, magnesium oxide or activated alumina. The first two proved the most suitable.

When also paper chromatography was used, the chromatogram was run on WHATMAN's paper No. 1 ascending with petroleum ether or methanol, or a mixture of both. The techniques were similar to those used by BAUER (1952) and GRANGAUD and GARCIA (1952). The absorption spectra were measured with BECKMAN's photoelectric quartz spectrophotometer.

Crystals of saponified carotenoids sometimes appeared already at the petroleum ether-methanol interphase. Usually crystals were obtained from evaporated residues of pigments. Owing to the small quantities of pigments in separate analyses of extracts from single specimens the recrystallizations were often practically impossible. For this reason the isolated carotenoid substances were relatively crude.

All procedures were performed in a N_2-atmosphere.

RESULTS

a) Fractionated extraction.

There was no difficulty in extracting the pigments from the soapy methanol by the first 3—5 petroleum ether fractions. These fractions were always of a greenish-yellow colour. Simultaneously with the advancing dilution of methanol by destilled water and the resulting precipitation of the soap the hypophase became more and more turbid. The extraction of pigment by the following petro-

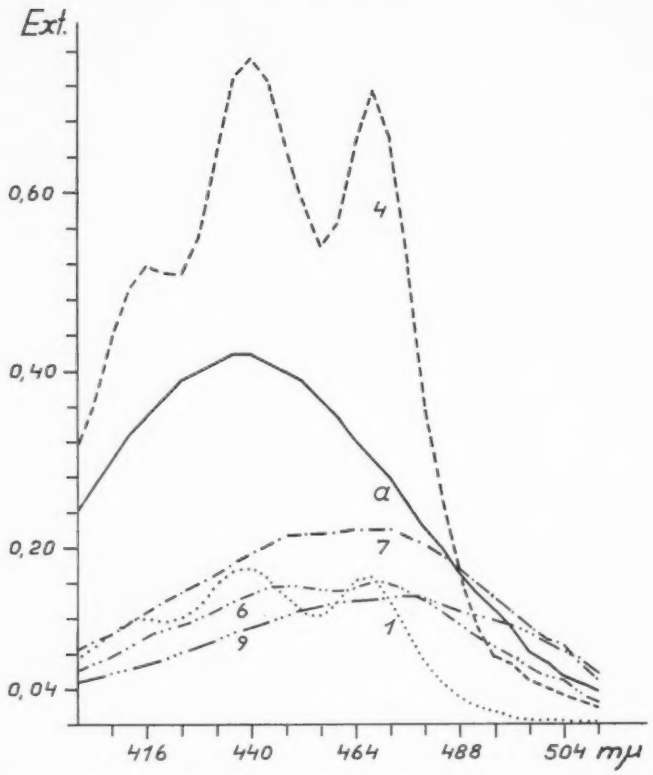

Fig. 1. Absorption spectra of saponified *Labrus mixtus* skin carotenoid fractions extracted from the hypophase by petroleum ether. 1—9 — fractions; a — azobenzene standard curve.

leum ether portions 4—6 required now about 12 hrs in each case. The extracts of these fractions assumed a pure yellow, then a reddish-yellow tint. The remainder of the pigment was extracted by 3—4 additional petroleum ether portions (fractions 7—10). As already mentioned it was now necessary gradually to acidify the hypophase by the addition of required quantities of c. 10 % acetic acid. These last extract fractions had an orange-red tint. After having been washed and dried as usual all fractions were examined spectrophotometrically. Fig. 1 shows curves of substances obtained in the course of one such fractionation procedure. If compared with the curves obtained following to chromatographic analyses, the curves of the first and the last fractions can be considered curves of pure isolated carotenoids, whereas the curves of intermediary fractions are of mixed nature. Moreover, in spite of the acidification it was never possible to extract the entire amount of saponified pigment from the hypophase. The soapy alcohol always remained slightly colored, probably by the presence of astacin. On account of the mentioned defects the fractionated

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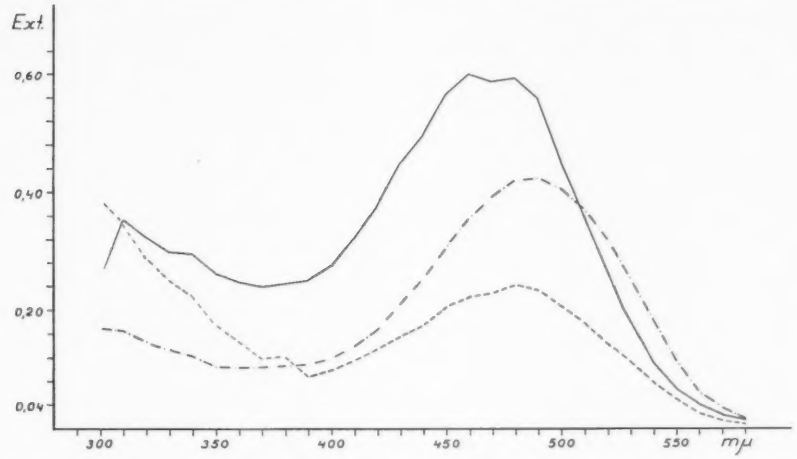

Fig. 2. Absorption spectra of unsaponified talc column eluates (astaxanthin ester), in pyridine, from extracts of the skin of *Labrus mixtus*. — and — — from male, — · — from female specimens.

extraction of carotenoids from the soapy hypophase can be accepted only as a simplified preliminary procedure which nevertheless can give reliable information about the nature of the pigments present.

b) Chromatographic partition.

By means of the chromatography on the column, which was the general method used in our work, one carotenoid substance was established in the sucrose eluate fraction, and another in the percolate fraction. This result was obtained both with crude and with saponified skin extracts. This permits the conclusion that the skin and fins of *Labrus mixtus* contain two carotenoid esters. Also the paper chromatography revealed only two carotenoids in skin extracts of *Labrus mixtus*.

Figure 2 shows absorption curves of unsaponified male and female talc eluates in pyridine. Male extracts exhibit a wide absorption band between 460—495 mμ, and the female one between c. 470—510 mμ. In some extracts from males absorption was observed also at 310, 340, and c. 380 mμ. It is evident that, as in *Crenilabrus pavo* (Abolinš and Abolinš-Krogis, 1957), the talc eluate contains also here an esther of astaxanthin. The deviations from the typical absorption spectrum of astaxanthin are obviously caused both by the esterifications and by concomitant substances in our pigment preparations. It must also be mentioned that in *Crenilabrus pavo* (Abolinš and Abolinš-Krogis, 1957) the peak of the wide absorption band of female extracts was shifted towards the short waves in relation to that of male extracts. In *Labrus mixtus*, on the

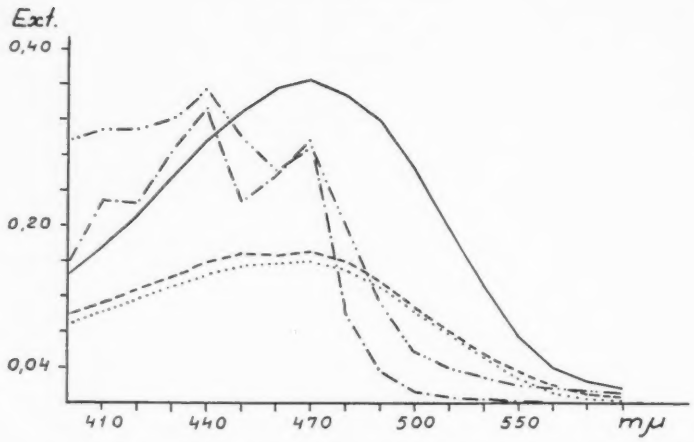

Fig. 3. — — and · · · Absorption spectra, in petroleum ether, of two *Labrus mixtus* female skin unsaponified talc column eluates (astaxanthin ester), — idem in petroleum ether, of a female skin saponified talc column eluate (astacin); — · ·— idem, in petroleum ether, of a female skin unsaponified talc column percolate (taraxanthin ester), — · — idem, in petroleum ether, of the saponified talc column percolate (taraxanthin) from the same specimen.

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contrary, the main absorption peak of the male extracts exhibited such shiftning. These differences were probably caused by different amounts of concomitant substances in our carotenoid preparations.

Upon the crystallization of sucrose (talc) eluates reddish-violet needles were obtained. Dissolved in pyridine they developed a slightly modified astaxanthin colour reaction if KOH was added in high vacuum, and if subsequently oxygen was admitted. Probably due to the presence of contaminations the developed colour was not clear blue, but violet, and changed into reddish.

Three one-top curves in Fig. 3 are from female eluates in crude and in saponified condition, all substances being dissolved in petroleum ether. The curve of the saponified eluate with a wide absorption band at c. 450—480 mμ is typical for astacin. As in extracts of *Crenilabrus pavo*, also here the absorption maximum of astaxanthin ester lies nearer the ultra-violet end of the spectrum than the absorption maximum of astacin.

Also the carotenoid substance which was obtained from sucrose (talc) percolate fractions was identical in male and female specimens. Fig. 4 shows curves of crude male and female percolates in petroleum ether. These percolates have been additionally filtered through zinc carbonate. Both curves have absorption maxima at 468, 440—436, and 416 mμ. In the Figure 5 the two uppermost curves have the same absorption maxima, yet they represent saponified percolates in petroleum ether. There was hardly any difference between the absorption spectra of the esterified carotenoid and the carotenoid itself in our percolate preparations. A similar identity of absorption spectra of percolate carotenoid ester

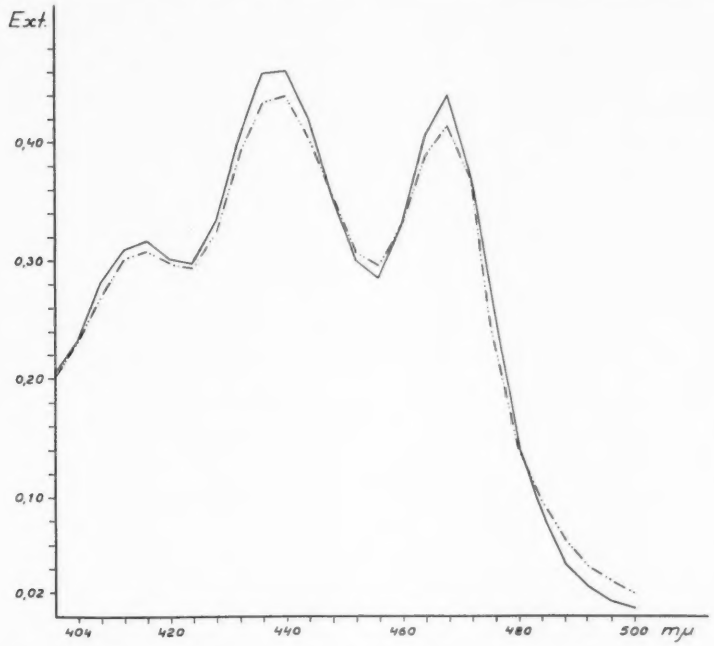

Fig. 4. Absorption spectra of unsaponified zinc carbonate column percolates (taraxanthin ester), in petroleum ether, from extracts of the skin and fins of *Labrus mixtus*, — from female, — · · — from male specimens.

and pure carotenoid was observed also for *Crenilabrus pavo* (ABOLINŠ and ABOLINŠ-KROGIS, 1957).

In order to determine whether or not the saponified sucrose percolate contained more than only one carotenoid, the male as well as female percolates were in some cases filtered furthermore through columns of calcium (zinc) carbonate. In such cases a very narrow deep orange adsorption zone was sometimes observed in the very top of the column. This zone was generally followed by a wide, rather diffuse yellow zone, but most of the pigment collected in the percolate. Already during the development of the column both zones moved downwards, and could be collected as the 2nd and 3rd percolate fractions. The three lowest curves of Fig. 5 refer to such fractions dissolved in ethanol. They are identical, and have absorption maxima at 472—468, 440—436, and 416. Some irregularity of the curve from the very narrow uppermost adsorption zone is probably due to a higher concentration of some contamination in it and to a very low concentration of pigment in the solvent. We must thus assume that the percolate fraction of *Labrus mixtus* skin extracts contains only one carotenoid. The absorption maxima of this carotenoid as well as of its ester nearly correspond to those of the taraxanthin: 471, 440 and 417 mμ (SMAKULA,

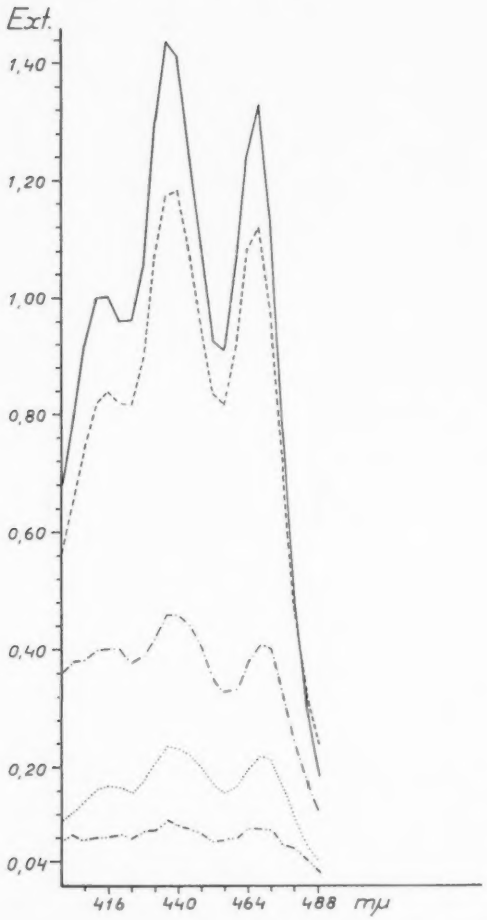

Fig. 5. — and — — Absorption spectra of *Labrus mixtus* male and female saponified talc column percolates (taraxanthin) in petroleum ether; —·— idem of male saponified talc percolate refiltered through zinc carbonate, percolate fraction; · · · and —·— eluate fractions (2nd and 3rd percolate fraction), all three in ethanol.

1934) or 472, 443, 417 mμ (GOODWIN, 1952), taraxanthin having in either case been dissolved in ethanol. In no case absorption values were observed which would correspond to those of lutein (xanthophyll). Also colour reactions and the form of crystals, where such were obtained, suggested the presence of taraxanthin. Yet until purified crystals are available we might prefer to use the term—a taraxanthin-like substance.

In some cases upon the partition of skin petroleum ether total extract with 90 % methanol traces of pigment were observed in the methanolic hypophase. Extracted once more with petroleum ether, and concentrated this trace-substance

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gave the same taraxanthin-like spectrum as the main pigment of the epiphase. It is still difficult to decide whether or not also a minute quantity of the unesterified carotenoid is present in the skin of *Labrus mixtus*.

Quantitative determinations of the carotenoid concentration in the skin were carried out with total extracts and with unsaponified sucrose percolate fractions. In the first case the sum of astaxanthin ester plus taraxanthin ester, in the second case the amount of xantophyll ester only were determined. Measurements were performed by means of Beckman's spectrophotometer. The calculations of carotenoid content were based upon comporison of the density of the unknown with the density of azobenzene standard (KUHN and BROCKMANN, 1932). Both, total extracts and percolates were calcuated as xanthophyll at the wavelength of 440 mμ, i.e. at the point of maximum absorption of percolates, and near the point of maximum absorption of total extracts. The amount of the caratenoid of the eluate fraction was not determined directly, but calculated as the difference between the amounts of total extract and percolate. The estimated content of carotenoids was set in relation to 1 g of fresh weight of the body or to 1 g of that of the skin and fins. Table 1 shows the results of quantitative assay.

The total content of carotenoids in the skin and fins was decidedly higher in females than in males, particularly if calculated in relation to skin (fins) weight unit. During spawning time the total carotenoid content in males varied between 48—81 μg per 1 g of skin freshweight, in females the corresponding figures were 72—93 μg. Measurements carried out about one month after spawning time gave lower values: 42—53 μg in males and 59—70 μg in females, yet one female exhibited a value as high as 97 μg. Observation of the visible intensity of body coloration in a larger number of male and female specimens led to the conclusion that this intensity varies in males much more than in females. The above quoted figures are from specimens with different visible intensity of body coloration and therefore reproduce to a certain extent the variation range of the total carotenoid content in the skin of *Labrus mixtus*.

Still larger sexual differences in carotenoid content are revealed by the comparison of taraxanthin and astaxanthin fractions. In male specimens the content of taraxanthin ester varied between 43—62 μg/g skin, in female specimens between 29—36 μg/g skin. Conversely, the content of astaxanthin ester amounted to 4.0—20.0 μg/g skin in males and to 42.0—56 μg/g skin in females. The ratio of astaxanthin ester was 59—64 % of the whole skin carotenoid amount in females and only 8.0—26.0 % of that in males.

In some specimens carotenoids were extracted separately from different regions of the skin and fins. Table 2 contains some figures expressing the distribution of both carotenoid esters over the body of the fish. Thus, in male specimen No 3 with an average taraxanthin ester content of 74 %, the skin of the head gave 80 %, the skin of the ventral region—70 %, the skin of the dorsal

TABLE 1.

Carotenoid content of the skin and fins of *Labrus mixtus* (estimated as xanthophyll)

Sample	Sexual condition and coloration	Fresh weight in g		Carotenoid content of skin and fins in μg per 1 g of body fresh weight					Idem per 1 g of skin/fins fresh weight				
		body	skin and fins	total carotenoids	taraxanth. ester	idem %	astaxanth. ester	idem %	total carotenoids	taraxanth. ester	idem %	astaxanth. ester	idem %
♂♂													
3	sp. typ. or.	252	34.0	10.1	7.4	74.0	2.6	26.0	76.0	56.0	74.0	20.0	26.0
4	„ „ „	279	30.6	8.9	6.8	77.0	2.1	23.0	81.0	62.4	77.0	18.6	23.0
6	„ „ yell.	237	25.8	5.3	4.7	89.0	0.6	11.0	48.0	43.3	88.0	5.0	12.0
9	af. sp. bl. yell.	284	36.8	6.0	5.6	93.0	0.4	7.0	47.0	43.0	92.0	4.0	8.0
14	„ „ or.	220	26.7	6.5					53.0				
22	„ „ bl. or.	290	33.3	4.8					42.0				
♀♀													
1	sp. br. r.	84	11.0	9.3	3.9	42.0	5.4	58.0	72.0	29.9	41.0	42.0	59.0
2	„ „ „	51	6.0	10.0	3.6	36.0	6.4	64.0	85.0	31.0	36.0	54.0	64.0
5	af. sp. r. yell.	98	11.4	10.7	4.2	39.0	6.5	61.0	93.0	36.5	39.0	56.5	61.0
15	„ „ r.	120	14.5	7.3					60.0				
16	„ „ „	71	9.3	8.9					70.1				
17	„ „ „	59	6.7	6.7					59.0				
18	„ „ „	79	8.0	9.8					97.0				

Abbreviations: sp. — in spawning; typ. — typical coloration; or. — orange; yell. — yellow; af. sp. — after spawning; bl. yell. — blue-yellowish; bl. or. — blue-orange; br. r. — brick-red; r — red.

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TABLE 2.

Carotenoid content in different fractions of the skin of *Labrus mixtus* (estimated as xanthophyll)

Sample	Skin fraction	fresh weight of fraction in g	Carotenoid content in µg per 1 g of skin or fins fresh weight				
			total carote-noids	tara-xanthin ester	idem %	asta-xanthin ester	idem %
3 ♂	head	8.9	24.0	19.0	80	5.0	20
3 „	ventral reg.	5.0	86.0	60.1	70	25.9	30
6 „	„ „	4.2	56.0	43.0	80	13.0	20
3 ♂	dorsal and lateral reg.	13.6	47.0	26.0	60	21.0	40
3 ♂	total skin	27.6	46.0	30.0	65	16.0	35
4 „	„ „	23.6	60.0	43.0	72	17.0	28
6 „	„ „	20.4	35.0	30.0	86	5.0	14
5 ♀	„ „	8.4	96.0	36.0	37	60.0	63
3 ♂	fins	7.3	190.0	160.0	84	30.0	16
4 „	„	7.0	156.0	128.0	82	28.0	18
6 „	„	5.4	100.0	91.0	91	9.0	9
5 ♀	„	3.0	80.0	39.0	49	41.0	51

and lateral regions—60 %, and the skin as a whole—65 %, whereas the fins gave 84 % of that ester. Obviously the prevalent yellow coloration of head, ventral region, and fins in males is due to the accumulation of taraxanthin ester in these regions. The absolute carotenoid content varies widely in different parts of the body of male specimens. The highest absolute content occurs in the skin of the ventral region, the lowest one in the skin of the head; the average absolute content of the fins is about twice as high as the highest one of the skin. For the females no such detailed evidence is available. Since in females as a whole astaxanthin ester prevails, the brick-red colour of the female body must be mainly determined by this carotenoid. In the female specimen No 5 the absolute content of carotenoids in the skin as a whole and in the fins lay within the same range. Also the distribution of two esters was nearly the same in the skin and in the fins.

DISCUSSION

The results obtained in the course of this investigation are in some respects similar to those obtained by the investigation into the skin carotenoids of *Crenilabrus pavo* (ABOLINŠ and ABOLINŠ-KROGIS, 1957), another fish of the family of *Labridae*. In the skin of the two *Labridae* only taraxanthin ester and astaxanthin ester could be established. Thus LÖNNBERG'S (1939) observation of both taraxanthin and xanthophyll (lutein) in the skin of *Labrus mixtus* could not be confirmed. Yet, a larger number of specimens from different localities might perhaps to be examined in order to arrive at a definite answer to the question whether or not lutein can replace taraxanthin in certain cases, or can exist together with it.

As in *Crenilabrus pavo* also in *Labrus mixtus* the male skin contains more aceton—and petroleum ether—soluble concomitant substances than that of the female. These substances have not been examined, but consist probably to a fair extent of sterols and fatty acids. Whether or not the different content of these substances in both sexes is related to quantitative sexual differences of carotenoid content, i.e. to the carotenoid metabolism, further research must show.

The carotenoid substances obtained after chromatography and crystallization were not recrystallized, and thus were not absolutely pure. The use of azobenzene, instead of pure taraxanthin and astaxanthin, as a standard substance in quantitative determinations probably also introduced some error into the figures of the carotenoid content which have been obtained.

The individual variation of the skin carotenoid content in correspondence to the observed wide individual variation of body coloration, must be important, particularly in males. The number of specimens examined in the course of this investigation is not sufficient enough to reveal the character of this variation. Yet specimens of quite different intensity of body coloration, especially in the case of males, have been chosen of the analyses. Our figures of the carotenoid amount per 1 g of body fresh weight and 1 g of skin fresh weight, respectively, are therefore to be regarded as single trial figures which illustrate the *ranges* of the true male and female carotenoid content. In spite of discussed limitations the existence of sexual differences of skin carotenoid total content, and still more of those of taraxanthin and astaxanthin content can be considered as beyond any doubt, since the corresponding male and female figures lie within very diverse ranges.

Neither in males nor in females could lutein, a derivative of α-carotene, be found. This means that *Labrus mixtus*, like *Crenilabrus pavo*, can accumulate only derivatives of β-carotene: the 3 : 3′ — dihydroxy — 5 : 6, 5′ : 6′ — diepoxy — β-carotene which is taraxanthin (GOODWIN, 1952) and the 3 : 3′ — dihydroxy — 4 : 4′ — diketo — β-carotene which is astaxanthin (KUHN and SÖRENSEN,

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1938). In males taraxanthin prevails, in females astaxanthin. The fishes live in the surf region at the depth of 5—12 metres, both sexes having access to identical food. It can hardly be imagined that the males prefer to choose food furnishing taraxanthin, and the females food supplying astaxanthin. Obviously both sexes dispose of divergent physiological mechanisms for the exploitation of the carotenoids of the food. Whether or not this means a different function of the gut, i.e. different carotenoid assimilation, different capacity of carotenoid oxidization, or different ability of carotenoid storage in the skin and different rate of carotenoid transformation into vitamin A, can not be decided yet, but it must be remembered that taraxanthin and astaxanthin are carotenoids of different oxidization degrees.

Apart from the high taraxanthin content and low astaxanthin content, the skin of males is characterized also by rich accumulation of a blue chromoprotein. In the skin of females the content of the blue pigment is relatively insignificant. This chromoprotein is a carotenoprotein. Its prosthetic group, as in other animal caroteno-proteins, is probably a carotenoid of the astaxanthin group. If this is so, the males of *Labrus mixtus* must in a much higher degree than the females possess the ability to convert the astaxanthin-type carotenoid into the carotenoprotein. Perhaps the low astaxanthin content in the skin of males is just the result of its partial conversion into chromoprotein. The typical male coloration pattern, inclusive of its blue component, is well developed only in mature males, and absent in young ones. Thus, it seems to be reasonable to continue the examination of all mentioned carotenoid components of the skin coloration pattern along the lines of a working hypothesis of a hormonal regulation of the realization of this pattern.

Although the sexual differences of the skin carotenoid content in *Crenilabrus pavo* (Abolinš and Abolinš-Krogis, 1957) are not so striking as in *Labrus mixtus,* a similar tendency can be observed in both. In both fishes the "pattern-bound male-characteristic carotenoid", as I might say, is the astaxanthin. Also in the red lateral pattern zones of the male *Crenilabrus* the percentage of astaxanthin is significantly higher than in correspondent zones of the females. The specific difference between the males of *Labrus mixtus* and those of *Crenilabrus pavo* is probably such that in *Labrus mixtus* the bulk of astaxanthin of the red lateral pattern is converted into the blue caroteno-protein, the lateral pattern becoming predominantly blue. In *Crenilabrus* obviously only a minor part of astaxanthin is converted into the chromoprotein, and the red colour of the pattern is preserved.

The male skin of *Labrus mixtus* accumulates, as we have seen, the taraxanthin in a much higher degree than the female one. This carotenoid is distributed over the whole skin of the fish with the highest concentrations ventrally, caudally, and in the fins. If the red pattern zones are excluded from the total of carotenoids, the taraxanthin prevails also in the skin of male *Crenilabrus pavo,*

being spread also in this fish over the entire body, but without significant concentration gradient. With regard to the general appearance of the fishes it seems permissible to call the taraxanthin "a diffusely distributed male-characteristic carotenoid".

What is now the true sexual difference in *Labrus mixtus* (and perhaps in the *Labridae* in general) as far as the skin carotenoid is concerned? To answer this question in detail, the mechanism of the production and deposition of the blue caroteno-protein in the skin of fishes must be further studied. The carotenoid content plus the caroteno-protein content mus be considered as a whole in both sexes. The ability of converting the astaxanthin into the caroteno- protein will perhaps prove to be the essential character of the male.

SUMMARY

The chromatographic and spectrophotometric analysis revealed the presence in acetone-pertoleum ether extracts from the skin and fins of *Labrus mixtus* of an astaxanthin ester and of a taraxanthin-like ester, both derivatives of β-carotene. As in *Crenilabrus pavo,* no lutein, a derivative of α-carotene, could be observed.

Quantitative determinations of the carotenoid content performed by comparing the optical densities of extracts with that of azobenzene standard, revealed important sexual differences. The total carotenoid content in females during the spawning time (72—93 μg per 1 g fresh weight of the whole skin and fins) significantly exceeds that in males (48—81 μg per 1 g). In males taraxanthin-ester (74—92 % of the total carotenoid content) is largely predominant, while in females astaxanthin ester (59—64 % of the total carotenoid content) prevails. In males the content of carotenoids is about twice as high in the fins as in the skin in general. In females the carotenoid content of the fins is of nearly the same range as that of the skin proper. The dominating brick-red colour of females is due to the presence of astaxanthin ester, while the typical orange-yellow tint of the ventral part of the body in mature males depends generally on the presence of taraxanthin ester. These sexual differences must be put in connexion with another sexual difference of the skin coloration in *Labrus mixtus,* viz. the high concentration of a blue caroteno-protein (probably an astaxanthin complex) in the male coloration pattern. The physiological background of the sexual differences of skin coloration is discussed.

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THE MICROSTRUCTURE OF DERMAL BONES IN ARTHRODIRES

BY

A. P. BYSTROW

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In 1954 I received some fragments of the bones of fossil vertebrates from Devonian deposits on Pioneer island (the Archipelago of North Land). Among the bones of different Middle Devonian fishes I have found several fragments with very unusual details. They could not be fragments of the armour of any known Devonian fishes. A detailed study of their microstructure has shown that they belong to three new forms of the Arthrodires.

I propose for one of them the name *Sedowichthys terrae-boreae* g.n., s.n. The genus is named in honour of G. J. SEDOW (1877—1914), a well-known Russian arctic explorer who perished in the Arctic in 1914; the species is named after the place where a fragment of the armour of *Sedowichthys* has been found for the first time the North Land (Terra Borea).

I propose for the second representative of the Arthrodira the name *Luetkeichthys borealis* g.n., s.n. The genus is named after F. P. LUETKE (1797—1882), a Russian arctic explorer.

I propose for the third new genus of the Arthrodira the name *Tollichthys polaris* g.n., s.n. in memory of E. TOLL (1858—1902), a Russian geologist and arctic explorer who perished in the Arctic at the end of 1902.

The study of the microstructure of the bones *Sedowichthys, Luetkeichthys* and *Tollichthys* has suggested to me to make a revision of the microstructure of the armour elements of several other Arthrodira as well. The present work is the result of all these investigations. All the illustrations in this work have been made by the author from his own preparations.

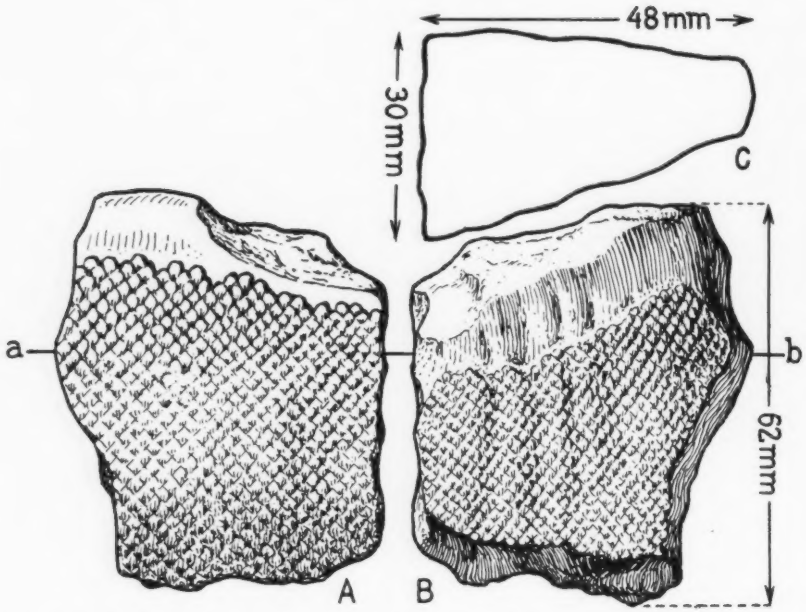

Fig. 1. *Sedowichthys terrae-boreae* g.n., s.n. Fragment of the armour. A — View from the convex side; B — view from the concave side; C — cross-section along the line a—b.

I. SEDOWICHTHYS TERRA-BOREAE g.n., s.n.

Only one fragment of the armour of *Sedowichthys terrae-boreae* has been found in Middle Devonian deposits on Pioneer island (Fig. 1). It is 62 mm in "length", 48 mm in "width" and its maximum thickness is 30 mm. One side of this fragment is convex (Fig. 1 A) and the other is concave (Fig. 1 B). In "transverse" section the fragment is wedge-shaped (Fig. 1 C), its one edge is 30 mm thick and the other—12 mm thick. This fragment is only a small part of the armour.

Nearly all the surface of the convex side of the fragment is covered with very unusual tubercles of a general rhomboidal shape (Fig. 1 A). These tubercles, like the scales in fishes, are arranged in oblique rows intersecting at an angle of 80°. A small part of the convex side of the fragment is not covered with tubercles and its surface is quite smooth. This undoubtedly proves that it never had tubercles.

Very indistinct "longitudinal" depressions can be seen on the convex sied of the fragment which is covered with a continuous layer of rhomboidal tubercles. These depressions become more noticeable only in those cases when the light falls upon the surface of the fragment at a very acute angle.

Under a magnifying glass the surface of the tubercles appears divided into

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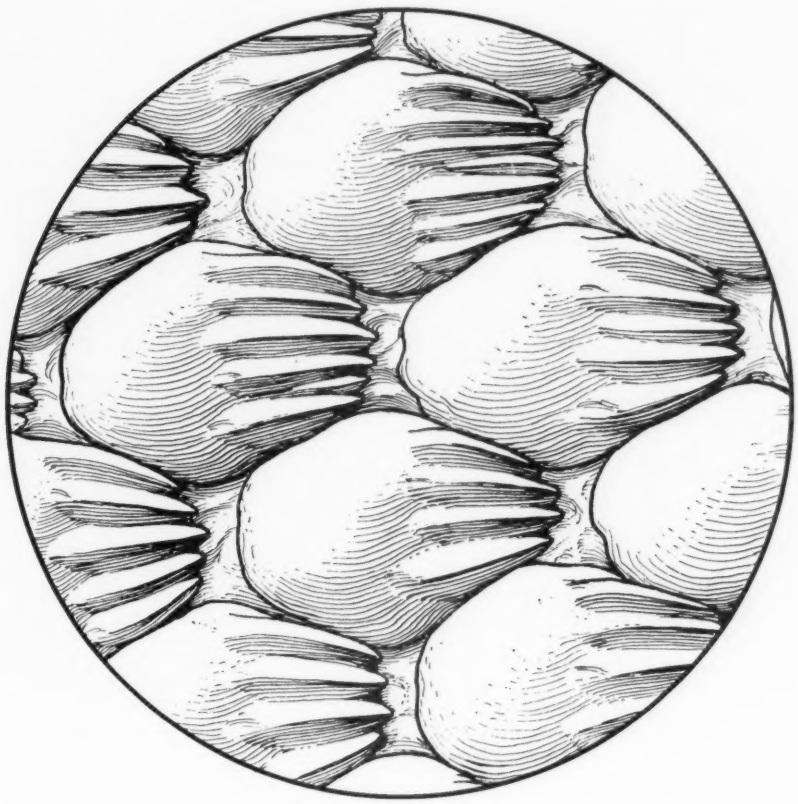

Fig. 2. *Sedowichthys terrae-boreae* g.n., s.n. Dermal teeth on the surface of the armour. × 25.

two sections of equal area (Fig. 2). The front section is usually convex and quite smooth while the back section is covered with high and sharp ridges. The number of these ridges varies from four to six or even seven. Usually these ridges are almost equal in length but sometimes a small ridge can be seen between a pair of well developed ridges. Its length may be half of that of the neighbouring ridges. The ridges gradually become lower towards the back part and terminate in spikes. The maximum length of every rhomboidal tubercle is 2 mm.

A greater part of the concave side of this fragment is also covered with rhomboidal tubercles (Fig. 1 B), and only a small part of its surface is free from them. The tubercles on the concave side of the fragment are smaller than those on the convex side and they have the same sharp ridges with ends having the same direction, i. e. directed caudalwards. The rows of the tubercles on the concave side also intersect at an angle of 80°. Several well defined "longitudi-

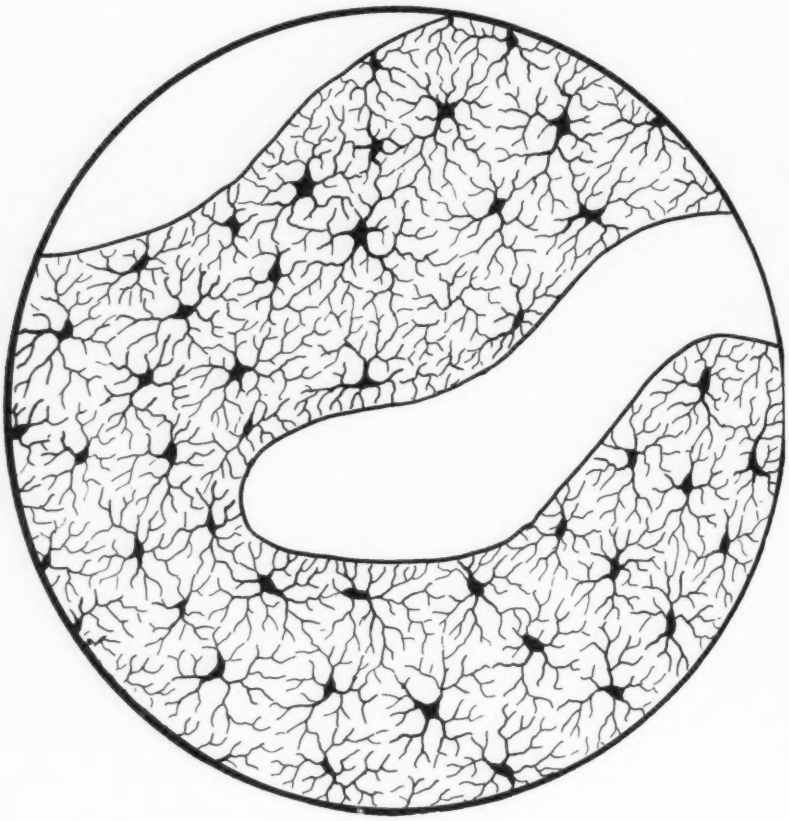

Fig. 3. *Sedowichthys terrae-boreae* g.n., s.n. Bone trabeculae in the middle of the armour. × 400.

nal" depressions can be seen on this side of the fragment both on the section covered with tubercles and on the smooth section.

The study of all the other sides of the fragment shows them to be fractured surfaces. They show very clearly numerous thin trabeculae and very narrow spaces between them filled up with the matrix.

Studying the fragment of the armour of *Sedowichthys terrae-boreae* which is at my disposal I have found it to differ greatly from the bones of all the other Middle Devonian vertebrates known to me. Owing to this the determination of the systematic position of *Sedowichthys* proved a very difficult task. This induced me to begin a close investigation of the microstructure of the armour of *Sedowichthys* using for this study a small piece which I have broken off from the unique specimen.

The microscopic investigation of the armour of *Sedowichthys terrae-boreae* showed first of all that the trabeculae are built of typical bone tissue. The

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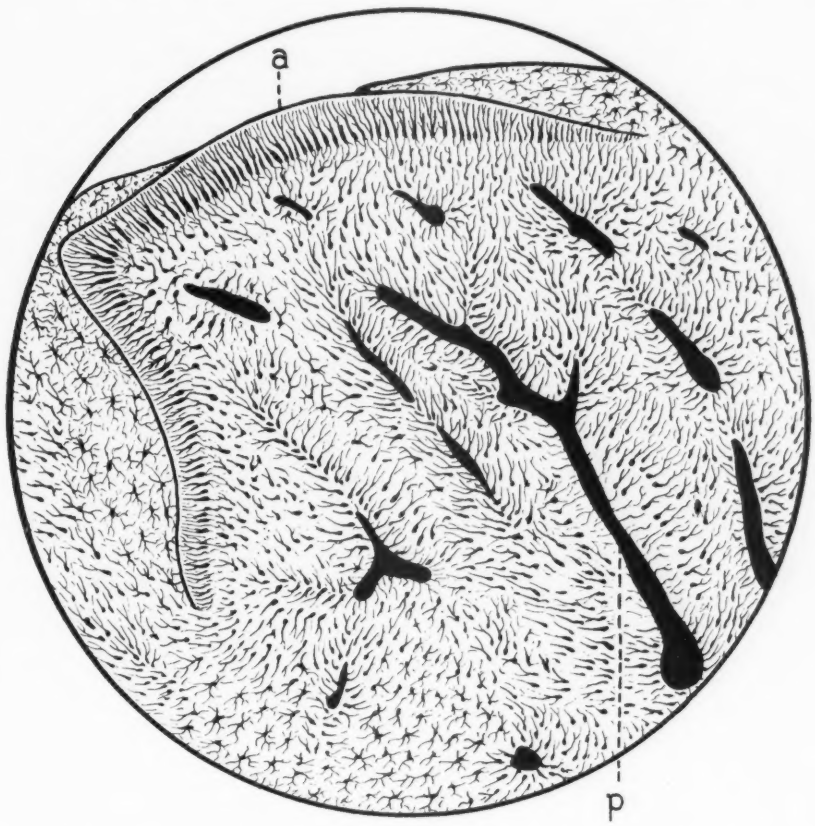

Fig. 4. *Sedowichthys terrae-boreae* g.n., s.n. Vertical section of a dermal tooth. a — wall of tooth; p — pulp canal. × 100.

transparent sections of these trabeculae clearly show numerous cavities of osteocytes (Fig. 3).

I could discover no traces of the rebuilding of the bone trabeculae in the armour of *Sedowichthys terrae-boreae*.

The microscopic study of the structure of the tubercles shows that in longitudinal section each of them is cone-shaped, this cone having relatively thick walls. This concical formation is strongly inclined backwards so that its sharp top is directed caudalwards (Fig. 4). One part of the wall of this inclined cone forms the upper wall of the tubercle. This wall occupies an almost horizontal position. Other parts of the wall of the cone form lateral sides of the tubercle. The back wall of the tubercle is slightly inclined and a little concave. The tubercle has no front wall, since the open base of the cone is directed towards the front.

A careful investigation of the microstructure of the wall of the tubercle has

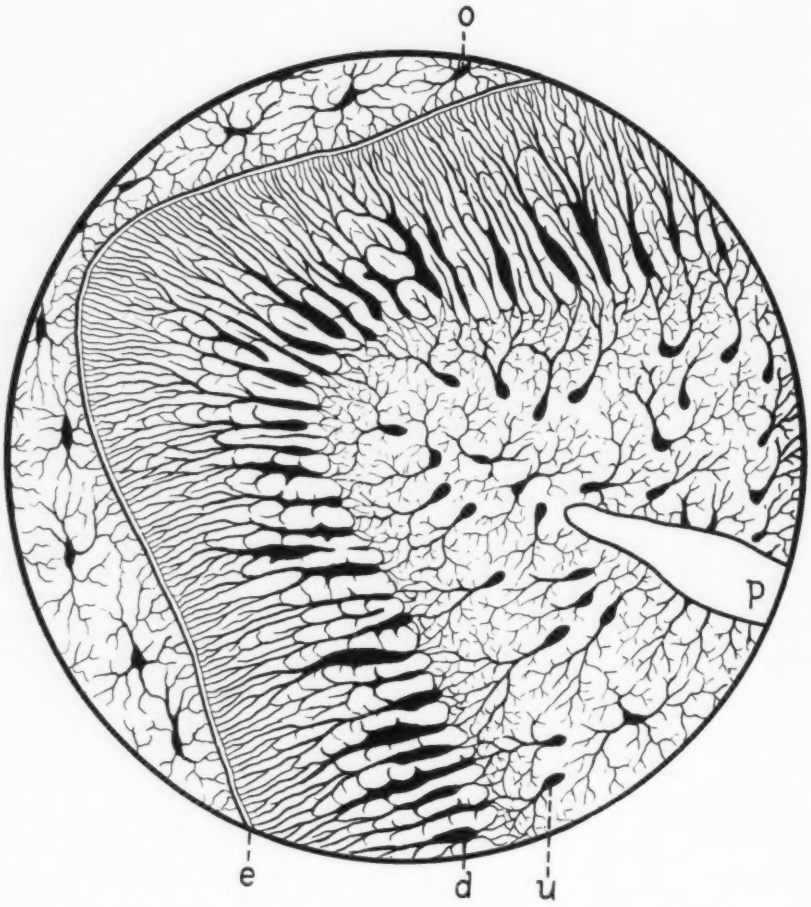

Fig. 5. *Sedowichthys terrae-boreae* g.n., s.n. Top of dermal tooth. d — cavity of odontoblast; e — enamel; c — cavity of osteocyte; p — pulp canal; u — cavity of unipolar cell. × 400.

provided very interesting evidence. The thick wall of the cone clearly seen under high magnification was found to consist of a transparent substance pierced by numerous canals (Fig. 5). These canals are orientated more or less perpendicularly to the surface of the wall. In the peripheral sections of the wall these canals are very narrow while in the basal section they are very wide. Both the narrow and the wide parts of these canals are frequently connected with the neighbouring canals by means of anastomoses.

The substance pierced by these unusual canals is dentine in which odontoblasts were immured. These cells were greatly elongated and were located in the wide basal parts of the canals (d - Fig. 6). Thin protoplasm processes of odontoblasts were located in the narrow parts of these canals.

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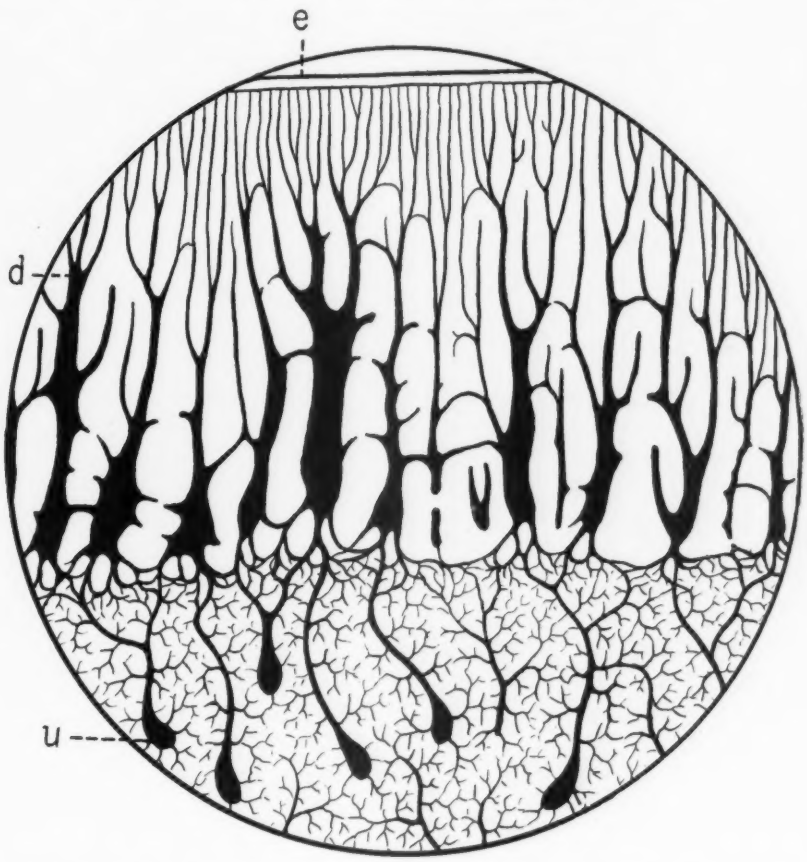

Fig. 6. *Sedowichthys terrae-boreae* g.n., s.n. Part of the wall of dermal tooth. d — cavity of odontoblast; e — enamel; u — cavity of unipolar cell. × 800.

The dentine wall is covered on the outside with a very thin layer of enamel. This layer can be readily seen in several parts, and the boundary between it and dentine is clearly defined (e - Fig. 5, 6).

Thus the walls of the tubercle on the bone of *Sedowichthys terrae-boreae* proved to be built of dentine covered with a thin layer of enamel. This fact means that every tubercle is a conical dermal tooth the sharp top of which is inclined backwards (caudalwards). The shape of this tooth is not a correct cone. On part of its dentine wall is flattened and disposed horizontally (a - Fig. 4). Large longitudinal ridges pass along this part of the wall (Fig. 2).

The pulp cavity of every such unusual dermal tooth was filled up with hard tissue. As a result it was transformed into a system of ramified and interconnected narrow pulp canals (p - Fig. 4).

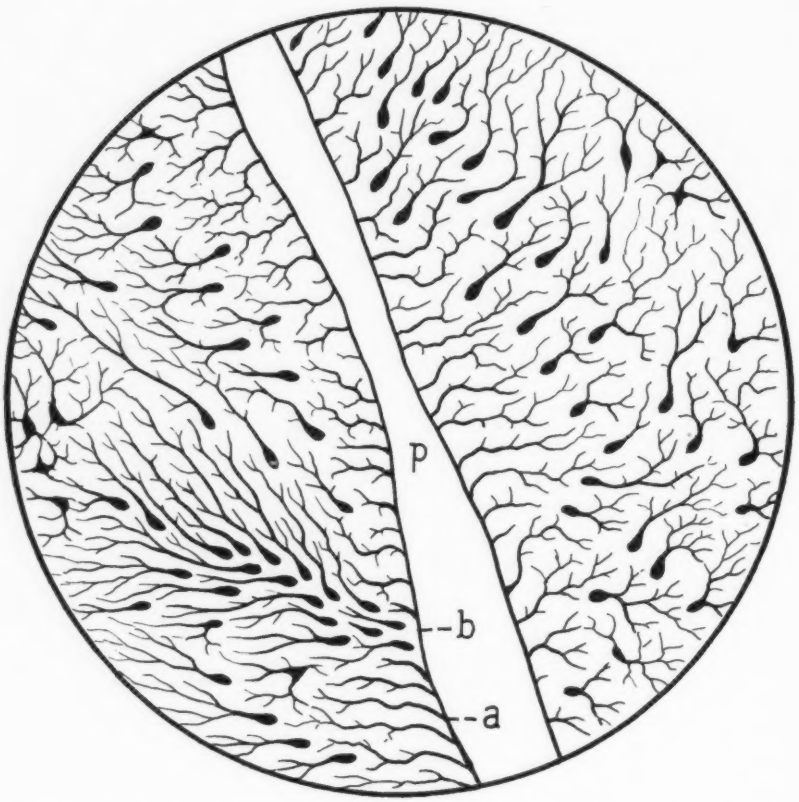

Fig. 7. *Sedowichthys terrae-boreae* g.n., s.n. Hard tissue filling up the pulp cavity of dermal tooth. a — canals of the processes of unipolar cells; b — accumulation of the cavities of unipolar cells; p — pulp canal. × 400.

The study of the hard substance filling up the pulp cavity of dental tooth in *Sedowichthys terrae-boreae* shows the structure of this substance to be very unusual.

Narrow canaliculi resembling dentine tubules of the trabecular tooth dentine in sharks branch off from each pulp canal (a - Fig. 7). During the lifetime of the fish these canaliculi were undoubtedly occupied by the protoplasm processes of the cells located on the walls of the pulp canals.

It would seem that these cells may be compared with the cells occupying the same position in the trabecular tooth dentine in sharks and may be called odontoblasts. However, the odontoblasts of trabecular dentine are never immured in dentine substance and always remain on the walls of the pulp canals.

The cells on the walls of the pulp canals in the dermal teeth of *Sedowichthys terrae-boreae* behaved quite differently. During the process of deposition of

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new hard substance layers on the walls of the pulp canals while the aperture of these canals narrowed more and more the cells on their walls were gradually surrounded by the hard tissue secreted by them and eventually were completely immured in it. The study of the structure of the hard tissue around the pulp canals in the dermal teeth of *Sedowichthys* shows these cells to be very unusual in shape. In most cases they were club-shaped and had one very well developed process. W. Gross (1930) has proposed for such cells the name "unipolar cells" (die Unipolarzellen). After some branch of the pulp canal in the dermal tooth of *Sedowichthys* had completely disappeared, only an accumulation of unipolar cells immured in hard tissue and orientated in one direction remained in its place (b - Fig. 7).

The main process of every unipolar cell has a great number of very thin lateral branches. The ends of these branches are connected not only with the ends of other branches of the same cell but also with the ends of a system of lateral branches of the neighbouring unipolar cells. As a result the hard tissue filling up the pulp cavity of dermal tooth in *Sedowichthys* is pierced by a dense network of extremely thin canaliculi. This network is very clearly seen under strong magnification (Fig. 8).

A dense network of anastomoses between unipolar cells greatly resembles the system of anastomoses between the osteocytes, and its character differs very widely from that of the anastomoses between the dentine tubules. Moreover, ordinary osteocytes can sometimes be found among a great number of unipolar cells (Fig. 7, 8). Therefore the hard substance filling up the pulp cavity of every dermal tooth of *Sedowichthys terrae-boreae* should be regarded as an unusual bone tissue and not as dentine similar to the trabecular tooth dentine in sharks.

The study of the boundaries between the dentine in dermal tooth and the bone tissue filling up its pulp cavity shows clearly that the basal parts of the cavities of the odontoblasts are connected by short anastomoses with the ends of the branches of the nearest unipolar cells (Fig. 6). The presence of such anastomoses explains in what way the nutritive substances could pass from the blood vessels present in the pulp canals to the odontoblasts immured in dentine.

So far as I know, the immurement of the odontoblasts in dentine has never been observed in any vertebrates. In *Sedowichthys terrae-boreae* this phenomenon was caused by the filling up of the pulp cavity of the tooth with bone tissue. As a result odontoblasts were completely immured in dentine without losing their connection with unipolar osteocytes.

The unusual bone tissue filling up the pulp cavity of every dermal tooth gradually passes over into typical bone tissue which constitutes the armour of *Sedowichthys*.

Ordinary bone is found also in the spaces between neighbouring dermal teeth. It connects the teeth in such a way that they form one compact layer. In

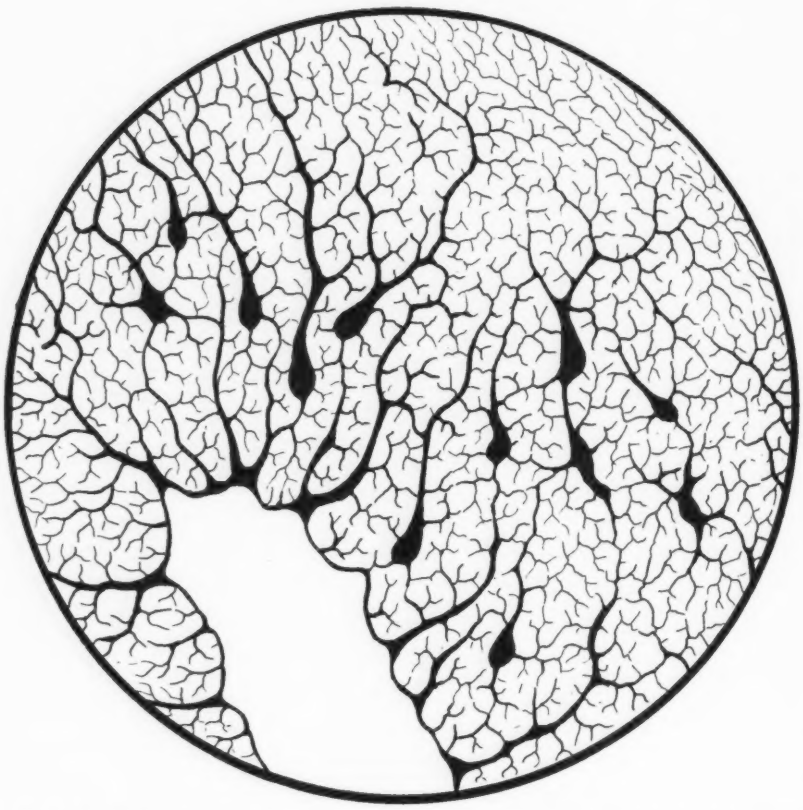

Fig. 8. *Sedowichthys terrae-boreae* g.n., s.n. Cavities of unipolar cells in the tissue filling up the pulp cavity of dermal tooth. × 800.

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some cases this typical bone tissue is also partially deposited on the upper surface of the tooth (Fig. 4, 5).

For solving the problem of systematic position of *Sedowichthys terrae-boreae* among fossil fishes the shape of the cavities of osteocytes in the bone trabeculae of its armour is not of great importance since at present we have no available evidence that would enable us to make use of the shape of osteocytes as well in solving taxonomic problems.

Nor can we use for this purpose the unusual structure of the dentine walls of dermal teeth in *Sedowichthys* since so far not a single fossil vertebrate with the odontoblasts completely immured in dentine has ever been described in paleontological literature.

As to the unipolar cells in the hard substance filling up the pulp cavity of every dermal tooth in *Sedowichthys,* such cells have already been found in the

armour of ancient fishes and therefore they can have a certain value in determining the systematic position of *Sedowichthys terrae-boreae.*

The cavities of unipolar cells in the armoure elements of fossil fishes have been described for the first time by W. Gross (1930). He discovered them in the tubercles on the bones of *Homostius sp., Coccosteus* (*Plourdosteus*) *livonicus* (Eastman), *Heterostius sp.* and *Macropetalichthys agassizi* (W. Gross, 1930, 1935). In 1933 A. Heintz discovered the cavities of unipolar cells in the tubercles on the bones of a Lower Devonian representative of the Order Arthrodira—*Phlyctaenaspis acadica* Whiteaves.

All these observations suggest that *Sedowichthys terrae-boreae* belongs to the Order Arthrodira.

I came to the conclusion that *Sedowichthys terrae-boreae* undoubtedly belongs to this order after a detailed investigation of the microstructure of the armour elements of several other Arthrodira (see below).

Taking into consideration the fact that the maximum thickness of some bones of *Sedowichthys terrae-boreae,* for example that of the fragment at my disposal, was 30 mm. One may say that this fish was of a very large size.

To determine the place occupied in the skeleton by this unique fragment of the armour of *Sedowichthys terrae-boreae* investigated by me is a very difficult task.

It could not be a part of any bone of the skull-roof since it was covered with dermal teeth on both sides.

It could not be a fragment of dorsal spine similar to that on the armour of *Rhamphodopsis trispinatus* Watson, 1938, since it has a convex and a concave side. The fragment curved in this way could be only a fragment of one a pair of bones—a right one or a left one.

One may assume that this fragment could be a part of a very well developed right or left projecting spinale. It is conceivable that the spinale of a primitive *Sedowichthys terrae-boreae* was as well developed as, for instance, that of *Arctolepis.* Only in such a position this fragment could be covered with dermal teeth on both sides and could be slightly curved. I cannot, however, say with confidence that such a localization of this fragment of the armour of *Sedowichthys* should be considered unquestionable. One objection against it is that this fragment has no cavity and is a solid mass while the spinale of the Arthrodires usually show a cavity.

The difficulty of solving the question of the localization of a small fragment of the bone of *Sedowichthys* in its skeleton is probably due to the fact that this fish was very unusual in structure which differed greatly from that of the known representatives of the Order Arthrodira.

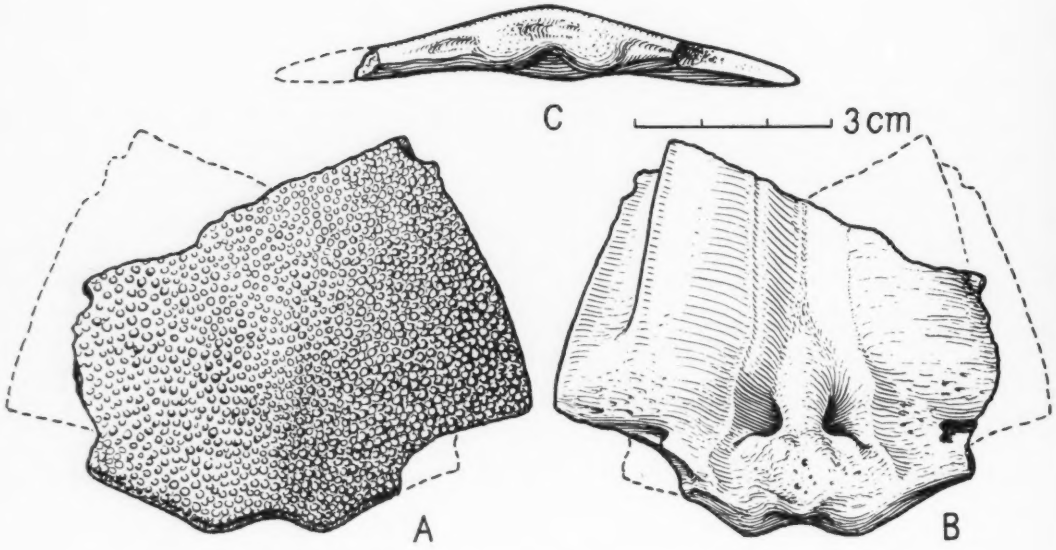

Fig. 9. *Luetkeichthys borealis* g.n., s.n. Incomplete nuchale. A — view from above; B — view from below; C — back view.

II. LUETKEICHTHYS BOREALIS g.n., s.n.

As pointed out above, I have discovered among the bone fragments of different fishes found in Middle Devonian deposits on Pioneer island (North Land) the fragments of the armour elements of a new representative of the Arthrodires for which I propose the name *Luetkeichthys borealis* g.n., s.n.

I had at my disposal nearly a complete nuchale of *Luetkeichthys*. The actual length of this bone cannot be determined because its front part is broken. The width of the bone is 8 cm, and its maximum thickness is 8 mm (Fig. 9). But one must bear in mind that this nuchale undoubtedly belonged to a relatively young specimen of *Luetkeichthys borealis*. This is borne out by the fact that small fragment of the same bone of *Luetkeichthys borealis* 13 mm in thickness have been found in the same deposits on Pioneer island.

The right and the left halves of the nuchale are disposed at an angle of 150° to each other (Fig. 9 C).

The outer surface of the nuchale is covered with numerous round tubercles 0,8 mm in diameter (Fig. 9 A).

A shallow longitudinal medial groove separated from the lateral sections of the surface by very indistinct ridges can be seen on the inner side of this bone (Fig. 9 B). Two rather deep pits are located on the lower surface of the bone. In shape and position they resemble similar pits on the corresponding bones of

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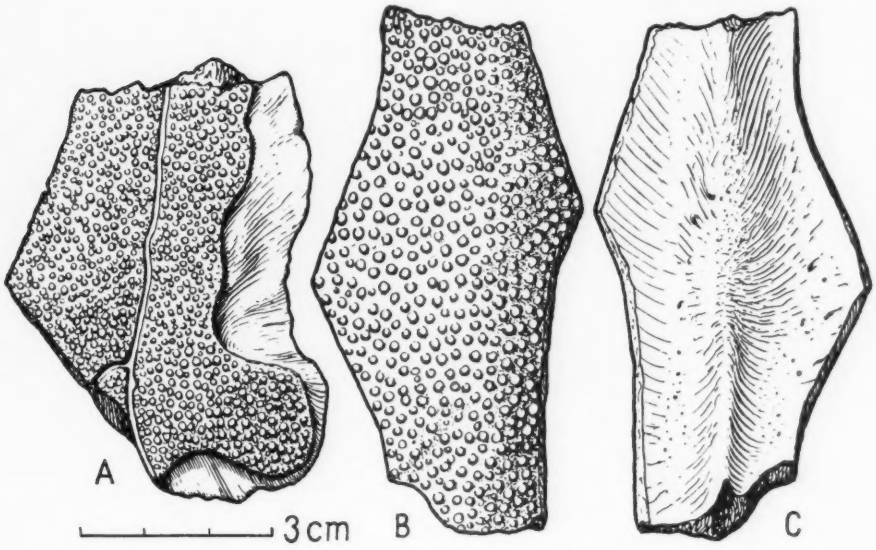

Fig. 10. *Luetkeichthys borealis* g.n., s.n. A — incomplete left marginale, view from above; B — fragment om mediodorsale, view from above; C — same fragment, view from inside.

Gyroplacosteus, Holonema, Plourdosteus (D. V. Obrutchev, 1931, 1933), *Heterostius* and *Coccosteus* (A. Heintz, 1934). Such strongly pronounced pits were absent on the nuchale of *Homostius sulcatus* (A. Heintz, 1934).

A greater part of the lateral sections of the nuchale in *Luetkeichthys* as in all Arthrodires overlapped the edges of the right and left paranuchale. As a result only a narrow band of the lower surface of the nuchale remained free in the place where a shallow longitudinal groove passed along this bone an a pair of pits was located. On the right and left edges of the nuchale of *Luetkeichthys borealis* there are slit-like depressions which received special sharp processes of the right and left paranuchale. In shape and in many detailes of structure the nuchale of *Luetkeichthys* differs from the corresponding bones of *Homostius, Heterostius, Coccosteus, Dinichthys, Holonema* and *Gyroplacosteus.*

Except a nearly complete nuchale and its small fragments, a sligthly broken left marginale of *Luetkeichthys borealis* has been found in the same deposits on Pioneer island (Fig. 10 A). This bone is similar in size to the corresponding bone of *Homostius sulcatus* (Kutorga), Heintz, 1934. However, the shape of suture separating this bone from the centrale and the paranchuale indicates that it could belong neither to *Homostius* nor to any other known large form of the Arthrodires.

A deep but narrow groove for the passage of the seismosensory canal, characteristic for this bone, passes on the outer surface of the marginale. Numerous round tubercles can be seen on the outer surface of the bone in

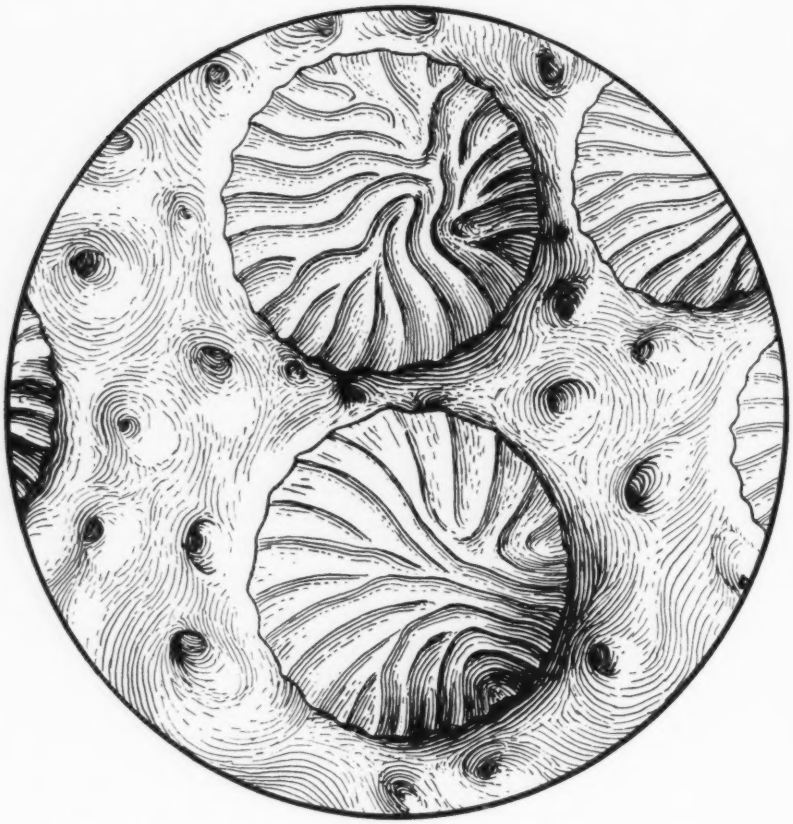

Fig. 11. *Luetkeichthys borealis* g.n., s.n. Dermal teeth on the surface of the armour. × 50.

those places where it was not overlapped by the edges of the adjacent bones.

A small fragment of the mediodorsale of *Luetkeichthys borealis* has been found in the same deposits as the nuchale and the marginale. The outer surface of the mediodorsale (Fig. 10 B) is covered with round tubercles 1,3 mm in diameter. This proves that this bone belongs to a more adult specimen of *Luetkeichthys borealis*. This latter speciment was larger than those represented by the nuchale and the marginale.

A longitudinal ridge runs on the inner side of the mediodorsale of *Luetkeichthys borealis* (Fig. 10 C); it is relatively high in the front and back sections and is lower in the middle section.

The study of the outer surface of the bones of *Luetkeichthys borealis* under strong magnification shows that the tubercles on this surface are covered with numerous sharp ridges (Fig. 11). These ridges are arranged more or less radially, they are usually curved and sometimes have lateral branches. Usually

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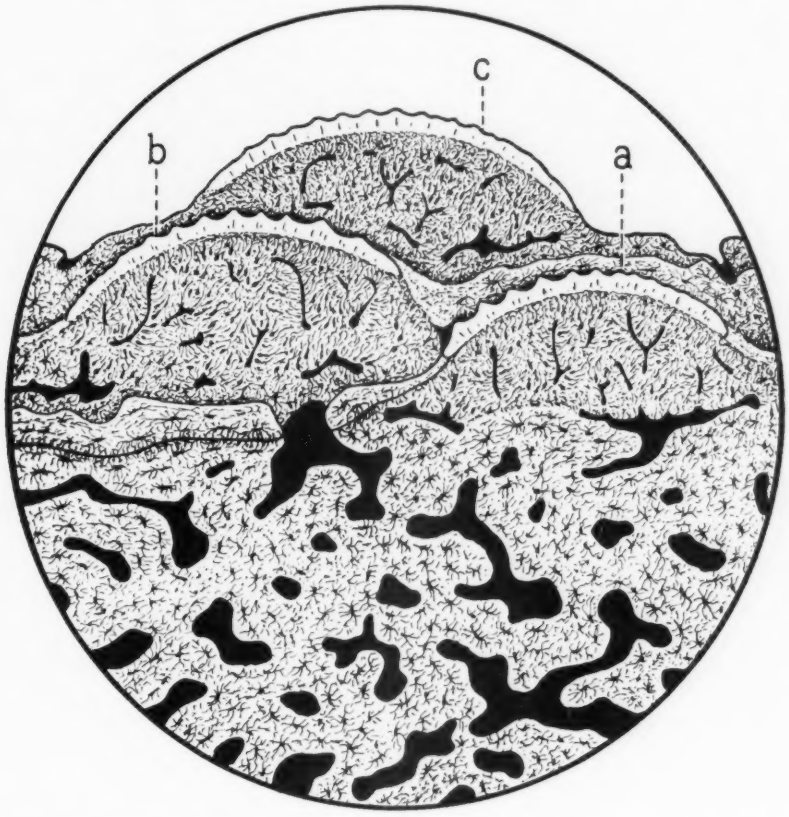

Fig. 12. *Luetkeichthys borealis* g.n., s.n. Vertical section of the armour. a, b, c — three consecutive generations of dermal teeth. × 50.

the centre from which these ridges radiate, when it is easily defined, is not located exactly on the top of the tubercle but is slightly displaced.

Numerous openings for the passage of the blood vessels can be clearly seen on the surface of the bone between these tubercles.

It is interesting to note that the same ridges as those on the tubercles of *Luetkeichthys borealis* have been discovered by E. WHITE (1952) on the tubercles of *Buchanosteus murrumbidgeensis* WHITE 1952, one of the representatives of the Order of Arthrodira found in Middle Devonian deposits in Australia. Unfortunately E. WHITE has not studied the microstructure of this form. Judging by the fragment of the skull found in Australia the size of *Buchanosteus* was twice smaller than that of *Luetkeichthys*.

The shape of the marginale in *Buchanosteus* greatly differed from that of the corresponding bone in *Luetkeichthys*. All this proves that *Buchanosteus* and *Luetkeichthys* are not identical forms.

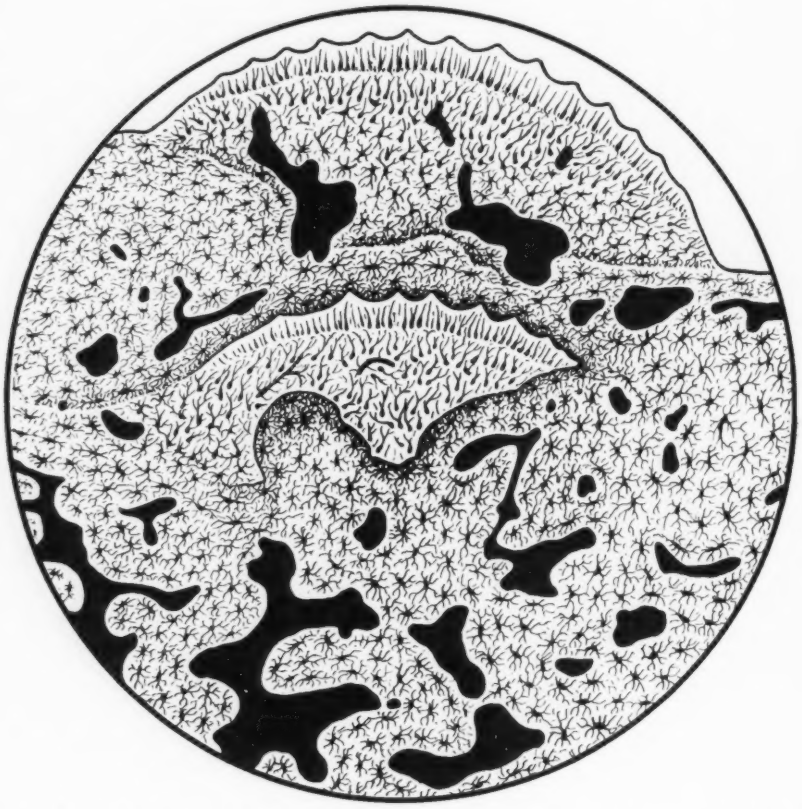

Fig. 13. *Luetkeichthys borealis* g.n. s.n. Absorption of dermal tooth immured in the bone (centre). × 100.

The microscopic investigation of the fragments of the skull of *Luetkeichthys* shaws their main mass to consist of typical bone. The vertical sections show that in some cases the tubercles are disposed in two or even in three layers. This is explained by the fact that on some parts of the bone the tubercles of one or two generations are immured in the bone substance (a, b - Fig. 12). In such cases only the tubercles of the last generation—a second or a third one, are found an the surface of the armour (c - Fig. 12). All the earlier generations of the tubercles are not preserved—they disappeared during the rebuilding of the upper layer of the armour. Sometimes the semi-destroyed tubercles of the earlier generations can be seen in tranparent sections (Fig. 13).

The tubercles of several consecutive generations differed in size: every tubercle of a new generation was always a little larger than of the preceding one. As a result the armour elements of more adult specimens of the fishes are covered with tubercles of a larger size than the armour elements of rela-

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tively young fishes of the same species. Thus the size of the tubercles enables us to determine the size and the relative individual age of fossiles fishes when we have at our disposal only small fragments of the armour elements belonging to different specimens of the same species. Incidentally, this enabled me to say confidently that the fragment of the mediodorsale at my disposal covered with large tubercles (Fig. 10 B) belonge to a more adult specimen of *Luetkeichthys borealis* while the marginale (Fig. 10 A) and the nuchale (Fig. 9 A) covered with small tubercles belong to a younger specimen of the same species. The microscopic structure of the tubercles on all these bones is identical.

Every tubercle on the cranial bones of *Luetkeichthys borealis* is covered with a layer of transparent hard substance (Fig. 12, 13). Sharp indentations can be seen on this layer. They are the cross-sections and the oblique sections of sharp ridges of the surface of the tubercles (Fig. 11).

The main mass of every tubercle consists of hard tissue pierced by narrow canals similar to the canals that could be seen in the bone substance filling up the pulp cavity of every dermal tooth in *Sedowichthys terrae-boreae* (Fig. 4).

The study of the microstructure of the tubercles of *Luetkeichthys borealis* under strong magnification shows that in the transparent layer covering them and forming ridges on their surface there are wide cavities passing over towards periphery into narrow canaliculi (Fig. 14). Both the wide cavities and the narrow canaliculi are quite identical with the similar formations in the dentine wall of dermal teeth of *Sedowichthys terrae-boreae* (Fig. 5, 6). This gives us a right to say that the tubercles on the bones of Luetkeichthys are covered with a layer of dentine.

In the dentine of both *Luetkeichthys* and *Sedowichthys* the odontoblasts occupied the wide cavities (d - Fig. 14) and only their thin protoplasm processes were located in the narrow canaliculi.

In *Luetkeichthys* the dentine layer is covered with a thin layer of enamel which even under very strong magnification can be seen only as a thin transparent stratum into which the ends of dentine tubules do not penerate (e - Fig. 14).

Thus the unusual tubercles on the dermal bones of *Luetkeichthys borealis* proved to be considerably modified dermal teeth.

The study of the microstructure of the main mass of every tubercle under strong magnification reveales the presence of numerous cavities of unipolar cells (u - Fig. 14). The unipolar cells of *Luetkeichthys borealis* are quite identical with similar cells in bone substance filling up the pulp cavities of dermal teeth in *Sedowichthys terrae-boreae,* and their thin processes form a similar dense network. Ordinary osteocytes can be found among the unipolar cells of *Luetkeichthys borealis* (Fig. 14) and moreover in a much greater number than in *Sedowichthys terrae-boreae.*

Thin branches of the unipolar cells lying in the immediate vicinity of dentine layer are connected with the odontoblasts by short anastomoses (Fig. 14).

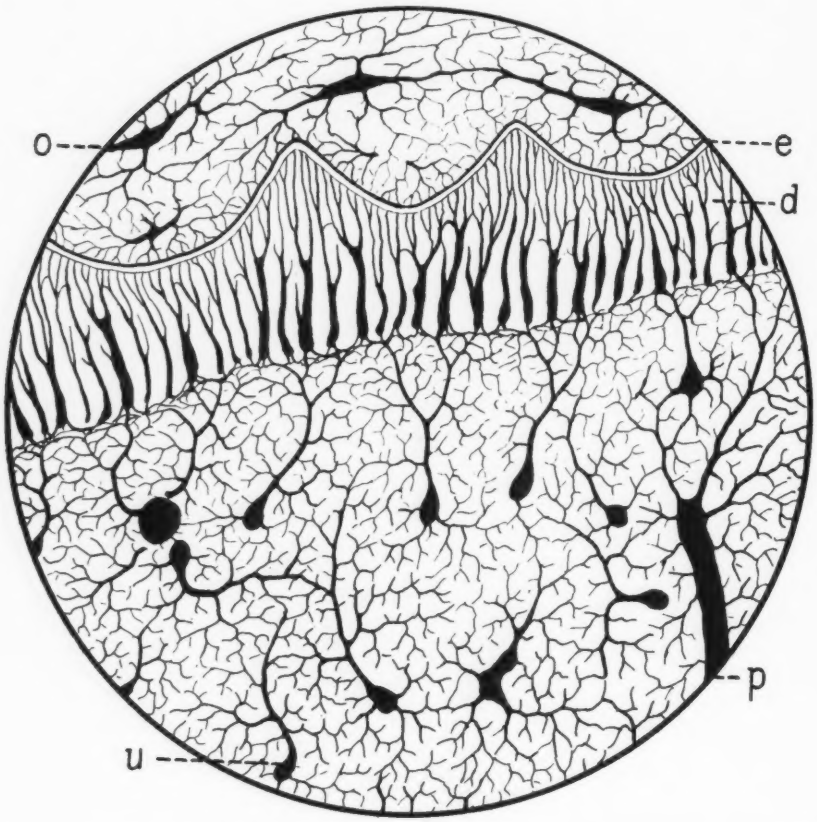

Fig. 14. *Luetkeichthys borealis* g.n., s.n. Part of the wall of dermal tooth. d — dentine; e — enamel; o — cavity of osteocyte; p — pulp canal; u — cavity of unipolar cell. × 600.

In the basal part of every tubercle the number of typical osteocytes among the unipolar cells gradually increases. As a result the unusual bone filling up the pulp cavity of dermal teeth passes over into ordinary bone that constituted the trabeculae of the middle layer of the armour.

In the lowest parts of the armour of *Luetkeichthys* the bone trabeculae are placed closer to each other, and the spaces between them are more narrow. However, the typical basal layer formed by the laminated bone mass is absent here.

Studying a great number of transparent sections of the bones of *Luetkeichthys* I have found several times the traces of the resorption of the early generations of the tubercles already immured in the bone but I could not find a single trace of the rebuilding of a system of trabeculae in the main body of the armour itself.

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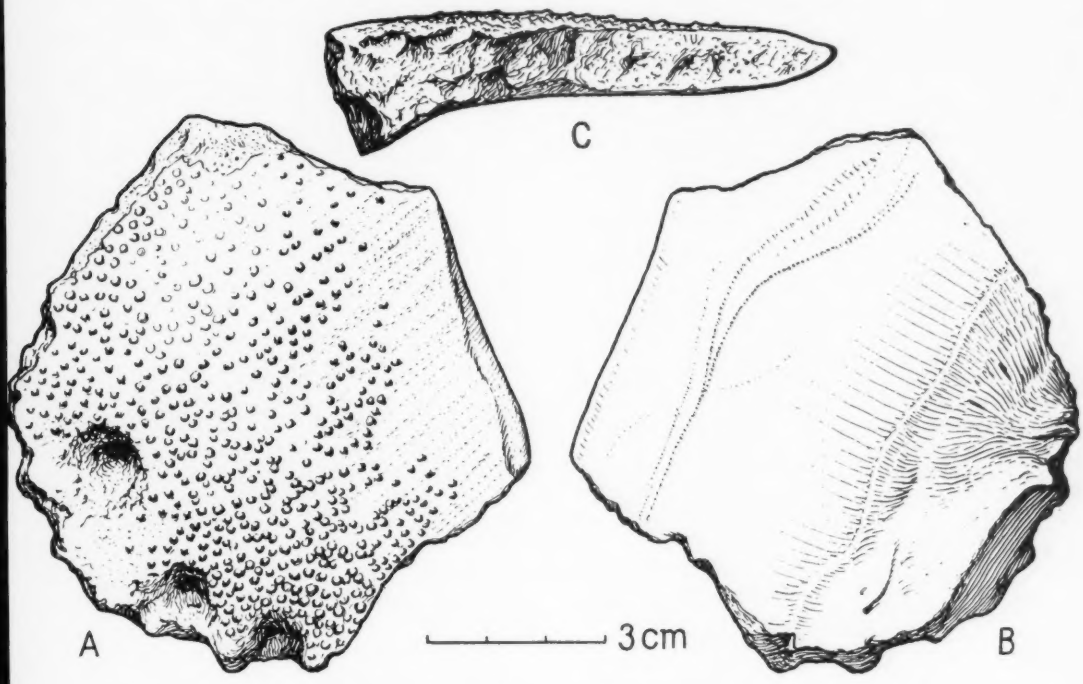

Fig. 15. *Tollichthys polaris* g.n., s.n. Fragment of the armour. A — veiw from above; B — view from below; C — lateral view.

A great resemblance of the microstructure of the dermal teeth of *Luetkeichthys borealis* to that of the dermal teeth of *Sedowichthys terrae-boreae* implies the existense of a relationship between these Middle Devonian fishes and shows beyond all doubt that *Sedowihthys* belongs to the Order Arthrodira.

Taking into consideration the fact that dermal teeth of *Luetkeichthys* have a hemispherical and not a conical shape we may say that this representative of the Arthrodires was a more progressive form than *Sedowichthys terrae-boreae*.

III. TOLLICHTHYS POLARIS g.n., s.n.

In the same Middle Devonian deposits on Pioneer island where the fragments of the armour of *Luetkeichthys borealis* have been found, have also been discovered several pieces of the skull of a third new representative of the Order Arthrodira—*Tollichthys polaris* g.n., s.n.

The largest fragment of the armour of *Tollichthys polaris* in some places has maximum thickness of 3 cm; its largest diameter is 9 cm (Fig. 15). One edge of the fragment is intact, all the others are broken.

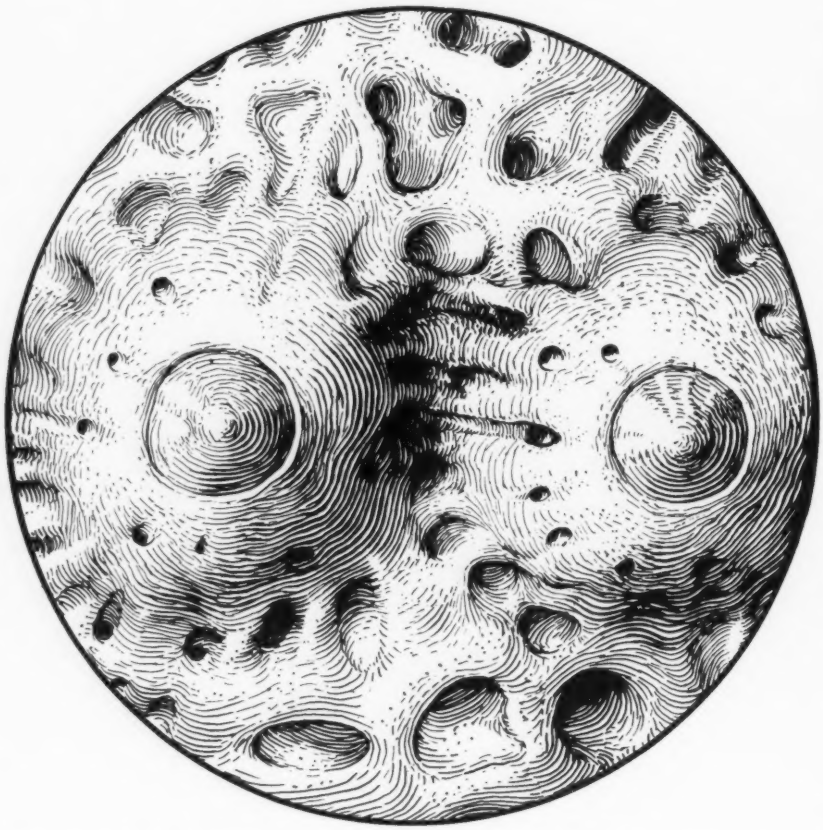

Fig. 16. *Tollichthys polaris* g.n., s.n. Tubercles on the surface of the armour. × 40.

The outer surface of the bones of *Tollichthys polaris* is covered with relatively widely scattered tubercles. They are disposed rather closely only in the area of the ossification centre of the armour element but become more scattered towards the edge of the bone. The tubercles are usually completely absent at some distance along the edge of the bones (Fig. 15 A). In some places the smooth inner surface of the bones shows grooves and openings for the blood vessels (Fig. 15 B).

Every tubercle on the bone of *Tollichthys* is shaped as a rather high cone. The base of some tubercles is 1,5 mm in diameter.

A round convex lamella commonly 0,5 mm in diameter is present on the top of every tubercle. The substance of the tubercle always sligthly overlaps the edges of the lamella as if moving upon it from the periphery (Fig. 16).

Numerous openings of different diameters for the passage of the blood vessels and nerves of the skin can be seen on the surface of the bone of

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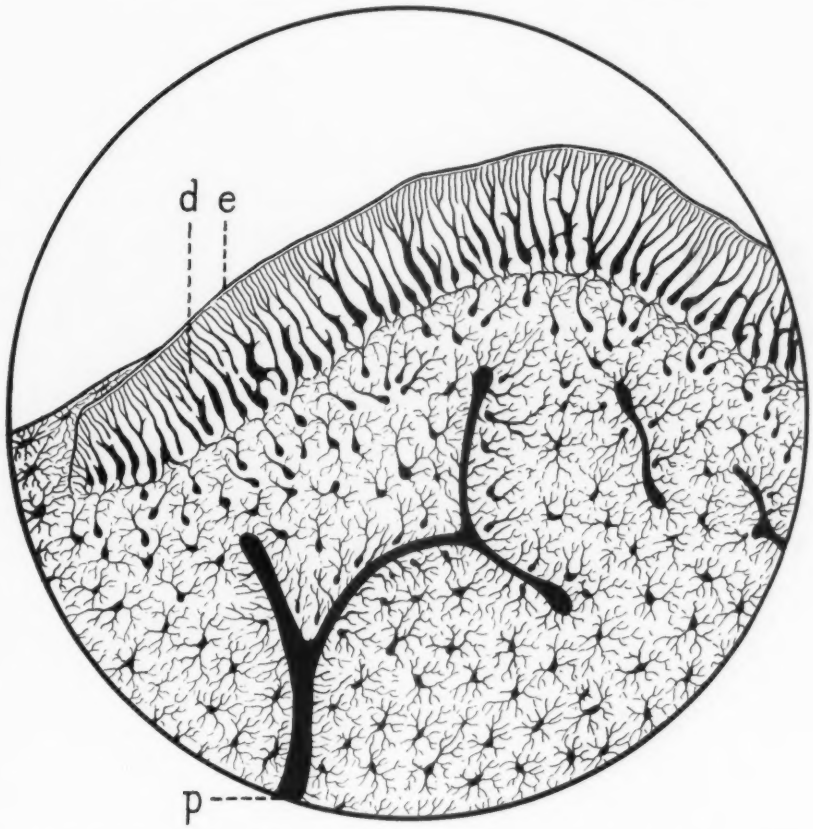

Fig. 17. *Tollichthys polaris* g.n., s.n. Vertical section of a tubercle. d — dentine; e — enamel; p — pulp canal. × 200.

Tollichthys polaris. Similar openings but of a much smaller diameter are located also on the slopes of the tubercles themselves.

The microscopic investigation of the transparent vertical sections of the tubercles of *Tollichthys polaris* shows that the round convex lamellae lying on their top consist of dentine. In every such lamella are located the cavities of elongated odontoblasts from which narrow dentine tubules branch off upwards. The cavities of the odontoblasts and the dentine tubules branching off from them are more or less perpendicular to the lower surface of the dentine lamella (Fig. 17). The anastomoses are to be seen between the neighbouring cavities of the odontoblasts and between the neighbouring dentine tubules (Fig. 18). The shape of the cavities of the odontoblasts in *Tollichthys polaris* (d - Fig. 18) is quite identical with the shape of these cavities in *Luetkeichthys borealis* (Fig. 14) and *Sedowichthys terrae-boreae* (Fig. 6).

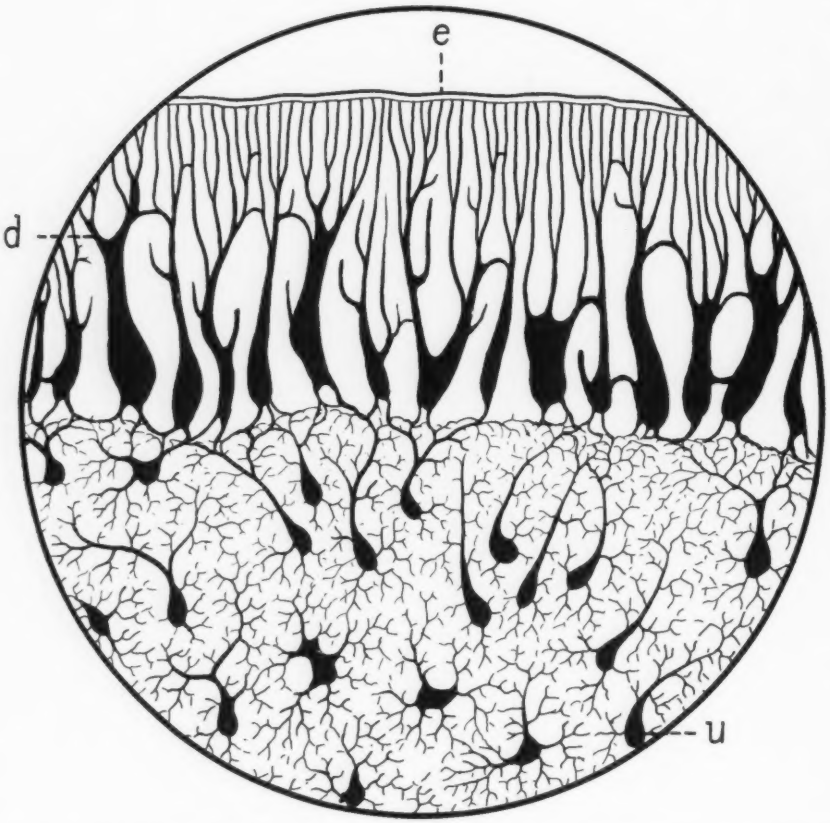

Fig. 18. *Tollichthys polaris* g.n., s.n. Part of the wall of dermal tooth. d — dentine; e — enamel; u — cavity of unipolar cell. × 800.

In *Tollichthys polaris* the dentine lamella on the tubercles is covered with a very thin but destinctly seen layer of enamel (e - Fig. 18).

The dentine-enamel layer on the tubercles of *Tollichthys polaris* is quite free from ridges. The main mass of every tubercle consists of bone tissue. The majority of the cavities of osteocytes in the tubercles of *Tollichthys polaris* have an ordinary shape but in the immediate vicinity of the dentine walls of the tooth the cavities of unipolar cells can be seen (Fig. 17). The thin branches of unipolar cells connected with each other form a usual dense network. This network is connected with the cavities of the odontoblasts by short but relatively wide anastomoses (Fig. 18) exactly in the same way as we could observe in *Luetkeichthys borealis* (Fig. 14) and in *Sedowichthys terrae-boreae* (Fig. 6).

The bone substance of the tubercles of *Tollichthys polaris* is pierced by a system of narrow pulp canals (p - Fig. 17).

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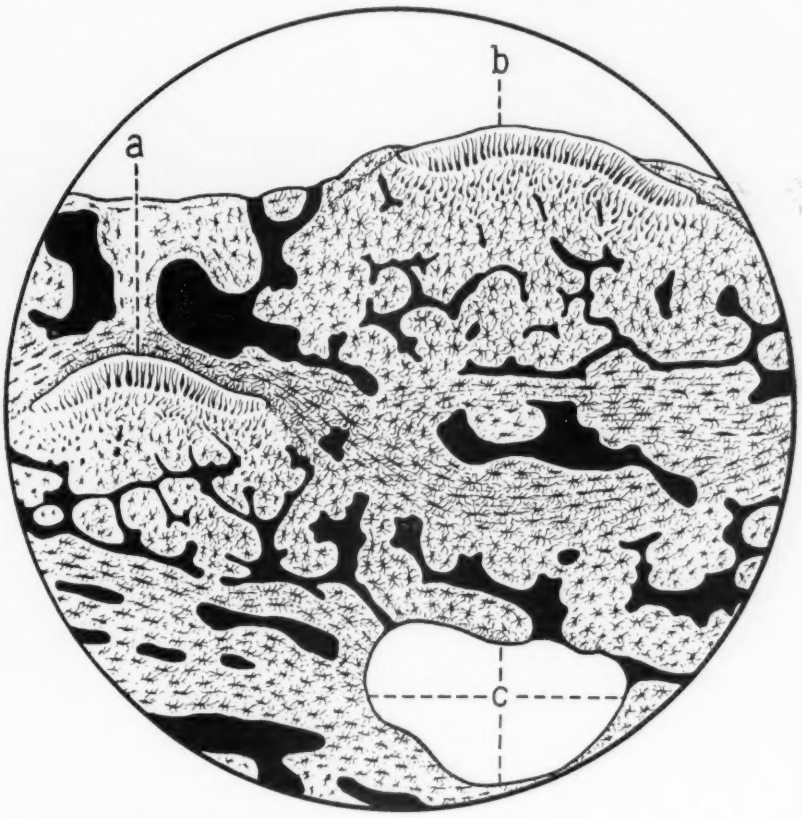

Fig. 19. *Tollichthys polaris* g.n., s.n. a — tubercle of an earlier generation; b — tubercle of a later generation; c — canal bored by some animal. × 100.

The transparent sections made of the parts of the armour elements located in the ossification centre usually show the early generations of the tubercles immured in the bone (Fig. 19).

The bone mass of the tubercles of *Tollichthys polaris* passes over without forming any distinct boundary into the bone trabecular forming all the main body of the armour. A compact basal layer is absent in *Tollichthys*. I could see no traces of the intensive rebuilding of the bone mass in the armour elements of *Tollichthys polaris*.

A great resemblance of many details of the microstructure of the tubercles of *Tollichthys* with the structure of dermal teeth of *Luetkeichthys* and *Sedowichthys* definitely implies that *Tollichthys* belongs to the Order Arthrodira.

Furthermore, the structure of the tubercles in *Tollichthys* clearly suggests that this representative of the Arthrodires was a more progressive form than *Luetkeichthys*.

IV. COCCOSTEUS

As we know, three layers can be distinguished in the armour elements of Coccosteidae.

1. The upper bone layer covered with tubercles and frequently containing the tubercles of the early generations immured in the bone.

2. The middle layer consisting of a spongy bone and formed by the irregularly disposed bone trabeculae.

3. The lower basal layer consisting of the horizontally lying bone lamellae.

The tubercles on the bones of the representatives of the family Coccosteidae show narrow canals which W. Gross (1935) has called "arcade-like" (Die arkadenförmigen Kanälen — W. Gross, 1935, p. 26). In their position and diameter these canals (p - Fig. 20) are identical with the pulp canals in the dermal teeth of *Tollichthys polaris, Luetkeichthys borealis* and *Sedowichthys terrae-boreae*.

Thin branches similar to dentine canals ramify from these "arcade-like" canals in the tubercles of Coccosteidae. W. Gross (1930, 1935) who has studied the microstructure of the bones of *Plourdosteus* (*Coccosteus*) *livonicus* (Eastman) writes:

„Oft sieht man auch direkt aus diesen Kanälchen zahlreiche Zellausläufer entspringen, die man ohne weiteres als Dentinröhrchen bezeichnen kann" (W. Gross, 1935 p. 26).

However, the character of the ramifications of these canals contradicts such an intepretation. In my opinion one may confidently say that these canals are not the dentine canals.

A certain number of cavities of unipolar cells can always readily be seen in the tubercles on the dermal bones of the Coccosteidae but at the same time the cavities of osteocytes of a usual shape are present here (Fig. 20).

The presence of a group of unipolar cells in every tubercle of *Coccosteus* suggested to W. Gross (1935) that these tubercles contain dentine. W. Gross writes:

„Die Unipolarzellenschicht entspricht in ihrem Aufbau dem Dentin des Zahntuberkels. Sie stellt eine Art Mischgewebe zwischen Knochen und Dentin dar, und ihre Zellen könnte man auch als Odontoblasten bezeichnen". (W. Gross, 1935, p. 26).

It is impossible to agree to this opinion. The unipolar cells are present in the tubercles on the bones of *Tollichthys polaris* (Fig. 18) in a greater number than in those of *Coccosteus* and are present in a still greater number in the tubercles of *Luetkeichthys* (Fig. 14). They occur in enormous quantities in the hard tissue filling up the pulp cavity of every dermal toots of *Sedowichthys terrae-boreae* (Fig. 5, 6, 7, 8).

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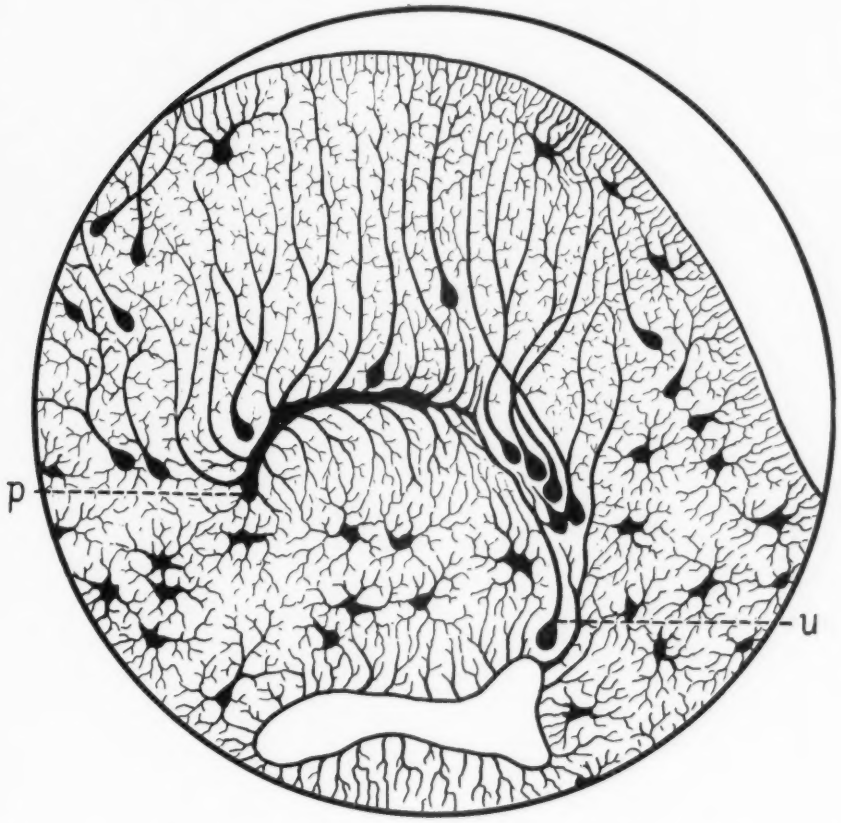

Fig. 20. *Coccosteus* sp. Vertical section of a tubercle. p — "pulp" (arcade-like) canal; u — cavity of unipolar cell. × 600.

As noted above, there is a dentine layer on every tubercle of *Tollichthys*, *Luetkeichthys* and *Sedowichthys*. Under the microscope this layer shows very distinctly the odontoblast cavities (Fig. 6, 14, 18). Therefore the study of the preparations readily shows that unipolar cells are always disposed under the dentine layer and cannot be regarded as odontoblasts.

During the initial stage of their formation the tubercles on the bones of Coccosteidae probably had relatively wide "pulp" canals. The unipolar cells were located an the walls of these canals and their long protoplasm processes deeply penetrated into the bone. While the unipolar cells were depositing layers of the bone substance on the walls of the "pulp" canals, the apertures of these canals gradually became more and more narrow. As a result the unipolar cells secreting the bone substance immured themselves in the bone. Such cells can usually be seen near the narrowed "pulp" canals.

Sometimes the tubercles of Coccosteidae show traces of completely immured sections of the "pulp canals. In such cases in the place formerly occupied by a "pulp" canal several cavities of unipolar cells lie side by side (Fig. 20). These cells had doubtless been located on the walls of the canals before the aperture was closed.

A close investigation of the microstructure of the armour elements of different representatives of the family Coccosteidae enables us to say that the tubercles covering the bones of these fishes show neither the cavities of the odontoblasts nor the dentine tubules. The surface of the tubercles of Coccosteidae is devoid of dentine and enamel.

W. Gross in his work on the teeth of the Acanthodii and the Arthrodira publisher in 1957 seems inclined to reject his former interpretation of the cell elements in the arthrodires. Thus he now calls the unipolar cells in the tubercles of *Actinolepis tuberculata* Agassiz the "Unipolaren Knochenzellen" and not the odontoblasts as before. He writes (1957, p. 23): „Die Schicht mit unipolaren Knochenzellen ist in den Tuberkeln von *Actinolepis* sehr deutlich ausgeprägt, aber vielfach strahlen dentinröhrchenartige Ausläufer unmittelbar aus den Gefässkanälen".

T. Örvig (1957) supposes that the tubercles on the bones of the arthrodires have "semidentine".

The armour elements of Coccosteidae grew in thickness by means of the formation of new generations of tubercles on their upper surface as well as by means of the simultaneous deposition of the bone lamellae on the lower surface of the basal layer.

The formation of new generations of tubercles led to the immurement of the tubercles of the preceding generation and to the thickening of the upper layer of the armour. The deposition of new bone lamellae on the lower surface of the armour resulted in the thickening of its lower layer. However, the thickening of the upper and lower layers took place relatively slowly. It was due to the fact that the intensive rebuilding of the bone trabeculae in the middle layer of the armour usually spread into the lower parts of the upper layer and into the upper parts of the lower layer. Owing to this during the growth of the armour in thickness the thickening of the middle layer took place more rapidly than that of the upper and lower layers.

V. HOMOSTIUS SULCATUS (KUTORGA)

I had an opportunity to study in detail the microstructure of the armour elements of such a large representative of the Arthrodires as *Homostius sulcatus* (Kutorga), Heintz 1934. The material for this study was provided by the

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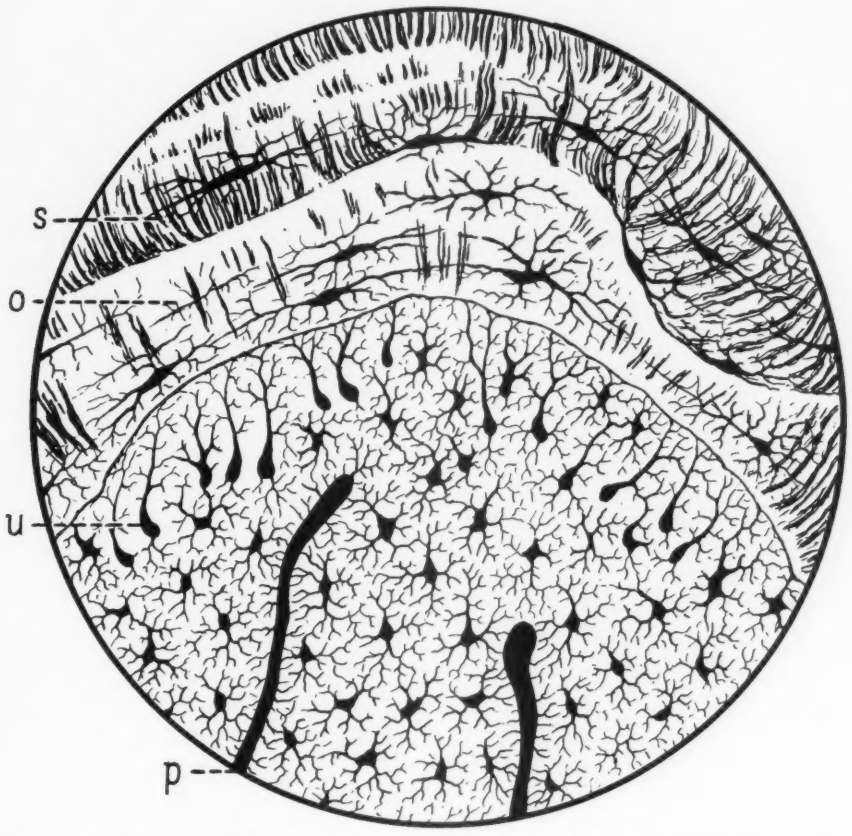

Fig. 21. *Homostius sulcatus* (Kutorga). Top of the tubercle immured in the bone. o — bone lamellae lying on tubercle; p — "pulp" canals; s — Sharpey's canals; u — cavity of unipolar cell. × 600.

bones of this fish found in Middle Devonian deposits in the neighboroughood of Tartu.

The armour elements of this representative of the arthrodires as well as those of Coccosteidae show three layers: the upper layer covered with tubercles and containing the tubercles of three or four generations immured in the bone, the middle spongy layer and a more compact and laminated lower layer.

In 1935 W. Gross discovered unipolar cells in the tubercles on the bones of *Homostius*. These cells, however, were mostly arranged neither in parallel nor vertically (W. Gross, 1935, p. 26).

A detailed study of the microstructure of the tubercles in *Homostius* shows that in this fish the unipolar cells occur only in the upper parts of the tubercles (u--Fig. 21). But here too side by side with these cells there are ordinary

osteocytes. The number of the unipolar cells in the tubercles of *Homostius sulcatus* is small; it is smaller than that in the tubercles of Coccosteidae. The main mass of very tubercle of *Homostius* consists of typical bone tissue containing ordinary osteocytes. But this tissue displays the same system of narrow "pulp" canals (p - Fig. 21) which was characteristic for such primitive arthrodires as *Sedowichthys, Luetkeichthys* and *Tollichthys.*

The surface of the tubercles of *Homostius sulcatus* is devoid of dentine. In order to be quite certain of it I have studied especially carefully the early generations of the tubercle completely immured in the bone tissue. Thus I could be sure that the surface of the tubercle had not been damaged during the preparation of the transparent section. Studying the structure of such tubercles I could in no case find any traces of that unusual dentine which had been so well known to me in the preparations of the bones of *Tollichthys, Luetkeichthys* and *Sedowichthys.*

A very interesting feature of the microstructure of the bones of *Homostius sulcatus* is the presence of a great number of Sharpey's canals in the bone lamellae lying immediately on the surface of the immured tubercles (o - Fig. 21). These canals are usually disposed more or less perpendiculary to the bone lamellae (s - Fig. 21).

Sharpey's canals are usually absent in the bone substance of the tubercles themselves.

The rebuilding of the armour elements of *Homostius sulcatus* occured most intensively in their middle layer. In this layer the newly formed bone lamellae are present everywhere replacing the resorbed parts of the bone trabeculae.

VI. HETEROSTIUS INGENS ASMUSS

I have studied the microstructure of the bones of *Heterostius ingens* Asmuss found in Middle Devonian deposits of the Estonian SSR. The armour elements of this very large representative of the Arthrodires are formed of three layers just as those of *Homostius.* The tubercles on the bone of this fish are built of bone tissue containing ordinary osteocytes. The unipolar cells occur among them very seldom.

W. Gross (1930) describing the unipolar cells in the tubercles of *Heterostius* notes that their orientations is dissimilar: in some of them the main process is directed upwards, in others—downwards and in the third—laterally.

All the bone mass of every tubercle is usually pierced by numerous canals most of which do not resemble the "pulp" or "the arcade-like" canals (Fig. 22).

I could find no traces of the dentine walls of dermal teeth on any tubercles of *Heterostius.*

The layer of bone covering the tubercles frequently shows numerous Shar-

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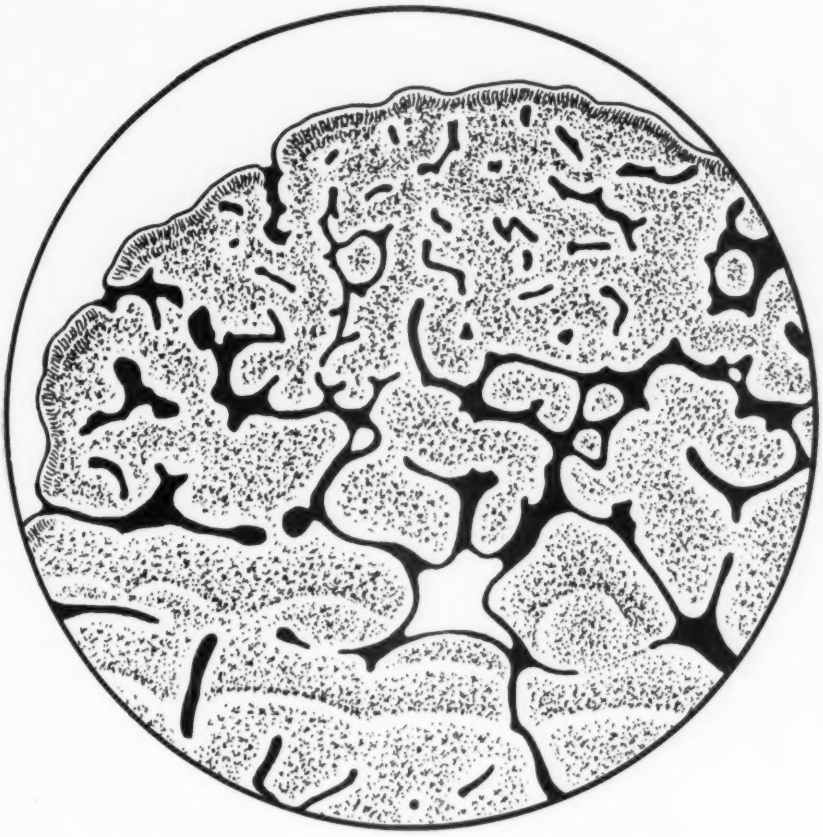

Fig. 22. *Heterostius ingens* Asmuss. Vertical section of a tubercle. × 50.

pey's canals directed perpendiculary to the surface of the tubercle (s - Fig. 23). Sharpey's canals are absent in the substance of the tubercles itself but they are very numerous in many bone trabeculae of the middle layer of the armour. This layer always shows very clearly the traces of the rebuilding of the bone.

VII. HOLONEMA RADIATUM OBRUTCHEV

The dermal bones of *Holonema* are formed of three layers: the upper layer consisting of the tubercles or ridges of different generations, the middle layer formed by spongy bone tissue, and the lower layer presenting the complex of horizontally lying bone lamellae connected with each other by bone trabeculae.

The microscopic study of the cross-sections of the ridges covering the armour

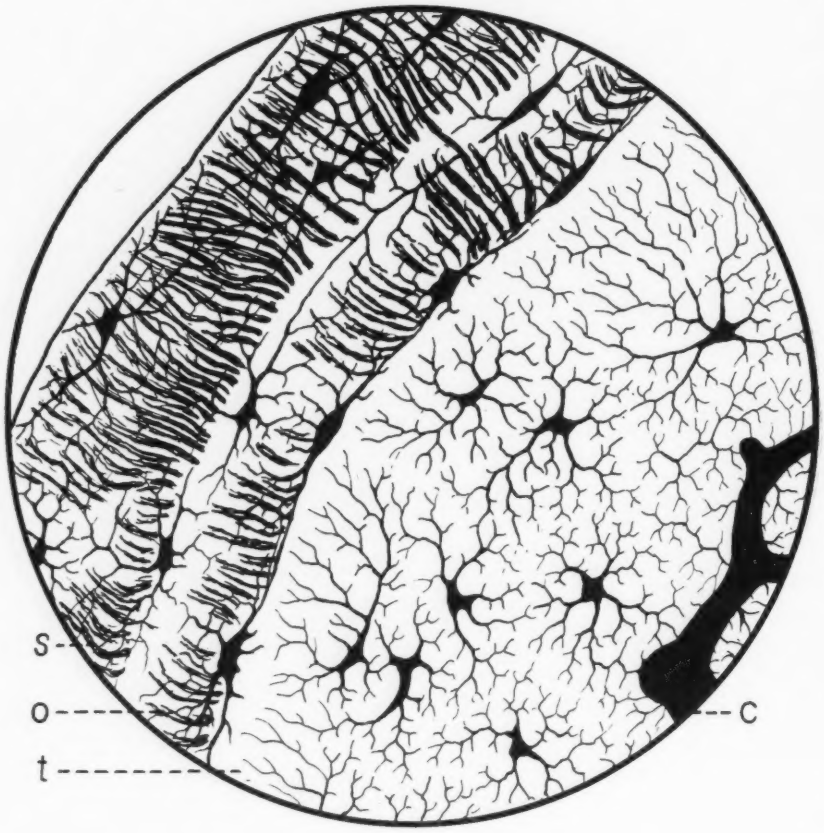

Fig. 23. *Heterostius ingens* Asmuss. Small part of a tubercle. c — "pulp" canal; o — bone lamellae; s — Sharpey's canals; t — tubercle × 600.

elements of *Holonema radiatum* Obrutchev, 1932, reveals only the bone tissue containing ordinary osteocytes.

I could not find typical unipolar cells in the ridges of Holonema, nor did D. V. Obrutchev (1932) in his studies of the microstructure of the bones of this representative of Arthrodira.

Of course there are no traces of dentine on the ridges of *Holonema*.

The surface layers of the ridges often show numerous Sharpey's canals orientated more or less vertically (s - Fig. 24). Sometimes these canals can be seen in the substance of the ridges itself.

Every ridge contains relatively wide cavities opening on the surface of the bone by means of special canals (c - Fig. 24). These cavities are quite different from the "pulp" or "arcade-like" (W. Gross, 1935) canals that are present in the tubercles of some other Arthrodires.

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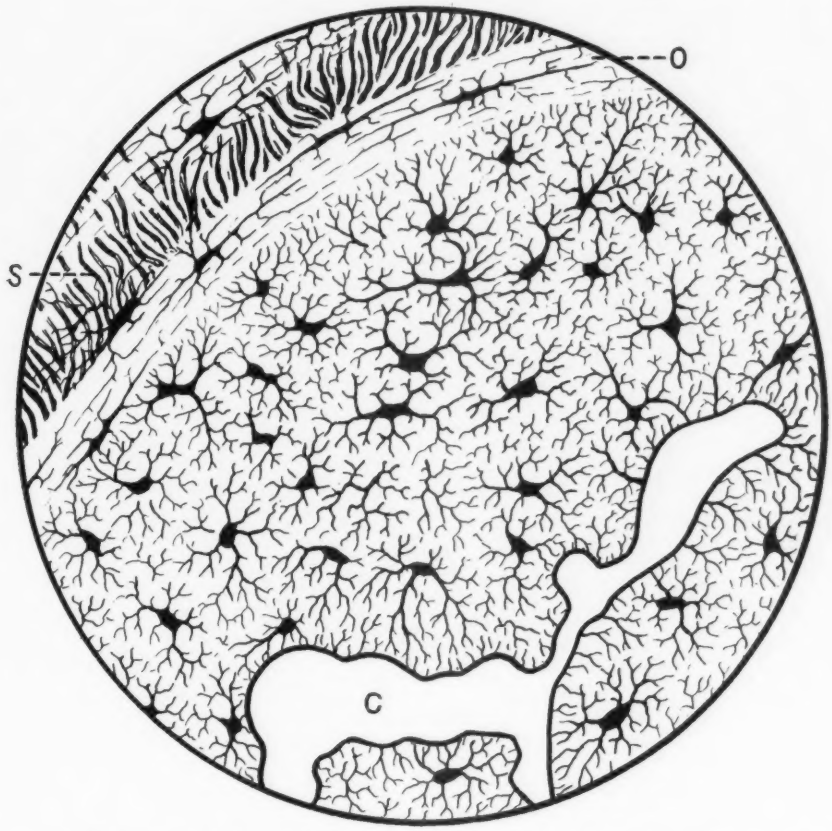

Fig. 24. *Holonema radiatum* Obrutchev. Part of a ridge in cross-section. c — canal; o — bone lamellae; s — Sharpey's canals. × 600.

The microstructure of the ridges of *Holonema* often shows traces of their rebuilding. In those cases new bone lamellae having a different orientation appear in place of the absorbed bone. Thus in *Holonema* one may observe the phenomena of the rebuilding of the superficial layer of the armour elements. But the middle layer of the bone undergoes the most intensive process of rebuilding. This layer often shows traces of repeated absorption and deposition of the bone lamellae.

The rebuilding spreads only to the upper lamellae of the lower layer.

VIII. PHYLLOLEPIS

As we know for a long time *Phyllolepis* had been considered an armoured jawless vertebrate. Only after the works of W. Gross (1934) and E. Stensiö

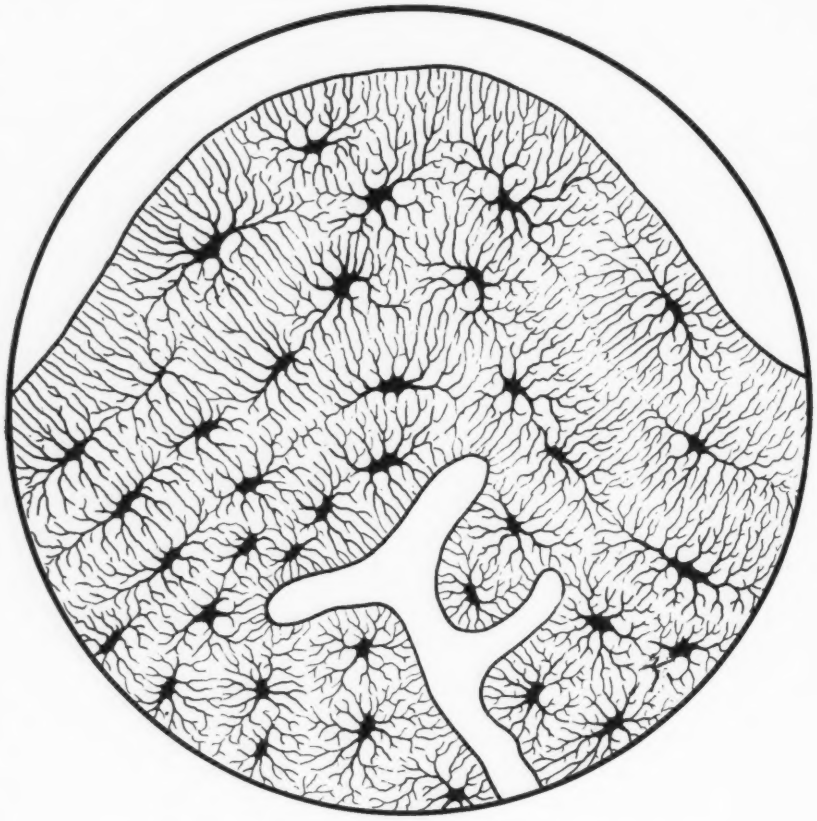

Fig. 25. *Phyllolepis* sp. Top of a ridge. Vertical section. × 400.

(1934, 1936) it became quite certain that this fossil animal belongs to the order Arthrodira.

The outer surface of the armour elements of *Phyllolepis* is covered with concentrically disposed ridges.

The microstructure of the bones of *Phyllolepis* has been studied by W. Gross (1934) and E. Stensiö (1936).

I have repeated the investigations of these authors on a fragment of the armour of *Phyllolepis sp.* from Lower Devonian deposits of the Leningrad district.

The armour elements of *Phyllolepis* clearly display three layers.

The upper layer forms high rather sharp ridges. These ridges consist of curved bone lamellae which contain large cavities of osteocytes with characteristic branches (Fig. 25). No traces of unipolar cells can be seen in the ridges of *Phyllolepis.*

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Every ridge shows relatively wide cavities. They are quite different from the "pulp" or "arcade-like" canals ("die arkadenförmigen Kanälen") present in the tubercles of other arthrodires.

The middle layer of the armour of *Phyllolepis* consists of bone trabeculae in which I could not discover any distinct traces of the rebuilding.

The lower layer is formed of horizontally disposed bone lamellae. These lamellae are separated by horizontal slits and are not directly adjecent to each other but in many places they are connected by the bone trabeculae. The basal bone lamellae and the trabeculae of the middle layer approximately equal in thickness.

IX. CONCLUSIONS

E. WHITE (1952) in his scheme of relationships and evolution of different groups of the arthodires derives these fishes from the presumably Silurian ancestors whom he calls unarmoured arthrodires (E. WHITE, 1952, p. 299).

Then, according to E. WHITE, at the end of the Silurian there appeared armoured arthrodires not yet found as fossils from whom the representatives of different families (super-family, after E. WHITE) and sub-orders of the Arthrodira are derived.

At present we have not got at our disposal such paleontological evidence that would enable us to reconstruct all the successive stages of the evolution of different forms of arthrodires. But it seems to me that the morphological material at my disposal enables me to determine a general outline of the changes which occured in the structure of the armour elements of these fishes during the process of their evolution.

The most primitive form of armoured arthrodires in all probability had relatively thin armour elements consisting of irregularly arranged bone trabeculae. The outer surface of these bones was covered with numerous dermal teeth with sharp tops directed caudalwards. It is quite possible that the pulp cavity of every such tooth was rather wide.

An example of such a structure of the armour elements may probably be found in the dermal bones of Lower Devonian *Stensiöella heintzi* Broili, *Nessariostoma granulosum* Broili and *Gemündina stürtzi* Traquair. E. WHITE places them in the Order Arthrodira (Class Arthrodires—E. WHITE, 1952).

In his work on the fishes of the Lower Devonian Broili (1933) writes that in *Stensiöella* all the armour lamallae on the head are covered with "nach rückwärts gerichteten Dornen" (F. BROILI, 1933, p. 290) and that in *Nessariostoma* every such thorn shows a cavity. F. BROILI has not studied the microstructure of these thorns but, at least in *Gemündina*, he regarded them as dermal teeth.

Later the armour elements in the more progressive forms of Arthrodires

acquired a much greater thickness but consisted as before only of bone trabeculae. The outer surface of such armour elements was covered with a compact layer of dermal teeth. The sharp tops of these teeth were inclined caudalwards and were a little flattened. A certain number of sharp ridges was formed on their flattened side. The large pulp cavity of every tooth was probably simultaneously filled up by unusual bone tissue containing unipolar osteocytes. This process transformed the pulp cavity into a system of narrow canals and made the dermal tooth a very strong structure.

This stage of development is represented by the armour elements of *Sedowichthys*.

During the next stage of the development of the arthrodires the structure of their armour elements remained essentially unchanged: they still consisted of the irregularly disposed bone trabeculae. As to the dermal teeth, they were greatly changed. As a result of these changes conical teeth acquired the shape of hemispheres. However, the surface of the dentine-enamel wall still retained sharp ridges. The pulp cavity of every such dermal tooth was filled up with hard tissue containing numerous unipolar osteocytes. During the growth of the armour of the fish in thickness such dermal teeth were immured in the bone tissue and subsequently destroyed during the rebuilding of the upper layer of the armour.

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Although the process of rebuilding took place in the upper layer of the armour, it did not spread to its middle and lower layers.

The dermal bones of *Luetkeichthys* may serve as an example of this structure of the armour elements.

During further development of the arthrodires a gradual reduction of the dermal teeth occured. During this process every dermal tooth took the shape of a very low rounded cone and the ridges on its surface were considerably flattened or even quite disappeared. Simultaneously with such a reduction of dermal teeth a gradual spreading out of the bone under them took place. As a result a rather high bone tubercle appeared with a rudimentary dermal tooth on its top. The cavity of this tooth was filled up with hard tissue containing unipolar cells and typical osteocytes. Narrow "pulp" canals were present in the bone basis of such a tooth. During the growth of the armour in thickness the early generations of the conical tubercles with rudimentary dermal teeth on their top were immured in the bone.

All the main mass of the armour elements consisted of the bone trabeculae which usually did not undergo the process of rebuilding.

This stage of evolutionary development is represented by the dermal bones of *Tollichthys*.

During the next stage of development the rudimentary dermal teeth on the bones of the arthrodires quite disappeared whereas the hard tissue which had filled up the pulp cavity of every dermal tooth was preserved. As a result

numerous tubercles appeared on the surface of the armour. Every such tubercle retained a certain number of the unusual unipolar cells characteristic of the hard tissue filling up the pulp cavities of dermal teeth in more primitive forms.

In spite of the complete reduction of the tooth wall on the surface of the tubercle a system of narrow "pulp" canals was retained in the tubercle.

Simultaneously with the complets reduction of dermal teeth three layers of different structure were formed in the armour elements of the arthrodires: the upper layer containing several generations of the tubercles immured in the bone, the middle layer formed by the irregularly disposed bone trabeculae, and the lower layer consisting of horizontally disposed bone lamellae.

The most active rebuilding usually occured in the trabecular system of the middle layer.

The armour elements of *Coccosteus* may serve as an example of this stage of development.

During the further evolution of the arthrodires the general structure of their armour elements remained essentially unchanged: they as before consisted of three layers. As to the tubercles, the number of the unipolar cells present in them dicreased and they were replaced by typical osteocytes. As a result there appeared the tubercles that contained a relatively small number of unipolar cells. These cells were usually to be seen only at the tops of the tubercles. In spite of such changes in the structure of the tubercles they still retained narrow "pulp" canals.

Such was, for instance, the structure of the tubercles on the bones of *Homostius*.

As a result of the following stages of the development of the bones the tubercles were now built mostly of ordinary bone tissue. The unipolar cells were seldom to be found among the osteocytes of this bone. Although every tubercle now was built of typical bone, it still retained the canals resembling the "pulp" canals.

In *Heterostius* one may find an example of this structure of the tubercles.

During the last stage of the evolutionary development of the bones of the arthrodires all the unipolar cells in the tubercles were replaced by typical osteocytes. Thus the tubercles now consisted only of ordinary bone tissue. Probably simultaneously or almost simultaneously all the "pulp" canals on the tubercles changed their diameter and their position and were transformed into relatively wide cavities. In some arthrodires such tubercles coalesced forming low ridges.

At this stage of development the armour elements as before consisted of three layers. Their growth in thickness took place by means of the deposition of new generations of tubercles or ridges from above and by means of the deposition of new bone lamellae from below. The rebuilding of the armour went on most actively in its middle spongy layer but partially spread to the lower and upper layers.

This structure of dermal bones may be observed, for example, in *Holonema*.

The material at my disposal gives only a certain idea of the evolutionary changes of the armour elements of the arthrodires and shows how the reduction of dermal teeth in the arthrodires proceeded.

But one must bear in mind that the complete reduction of dermal teeth was observed not only in the later progressive representatives of the Order Arthrodira the example of which may be found in *Coccosteus, Homostius, Heterostius, Holonema* and others, but also in such a primitive and at the same time specialized ancient form as *Lunaspis* of the Lower Devonian. The tubercles on the bones of *Lunaspis* are almost identical in structure to those on the bones of *Coccosteus*.

The study of the microstructure of the armour elements of the arthrodires doubtless provides a very interesting and valuable morphological material. However, this material alone is evidently quite inadequate for the determination of the evolutionary development of these fishes. To solve the complicated problems of the systematics, relationships and the evolution of the arthrodires a thorough and detailed investigation of the skeleton structure of these armoured fishes is necessary, in so far as the available material permits. In this investigation all the features of the microstructure of the bones should be taken into consideration as a necessary supplementary evidence.

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NOTES ON THE DISTRIBUTION OF THE MITOCHONDRIA IN THE YOUNG SPERMATIDS OF SOME MAMMALS

BY

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In the very young spermatids of insects the mitochondria assemble near the nuclear membrane to form a peculiar mitochondrial body, the "Nebenkern". This body later divides into two parts, which join the tail of the spermatozoon. The presence of a similar arrangement has not been reported for mammalian spermatids and was denied by MASUI (1923). However, mitochondrial aggregates were observed by the present author in early spermatids of stallions and rabbits, and a fresh study of the distribution of the mitochondria of the spermatids in some common domestic and laboratory animals was therefore undertaken.

MATERIAL AND METHODS

Material was collected from healthy stallions, bulls, rams, dogs, cats, rabbits, guinea pigs, rats, and mice. Small pieces of the testes were cut out immediately after the death of the animal, or after castration. Fixation was performed in Helly's fluid and/or Champy's fluid and was followed by treatment with potassium dichromate for 4 to 6 days. After embedding in paraffin wax sections were cut at 3 μ and stained with Heidenhain's iron hematoxylin, Kull's mitochondrial stain, and, after Helly's fixation, Cowdry's aniline fuchsin-methyl green. As post mortem changes are known to cause swelling and crowding of mitochondria, only the peripheral regions of the sections were studied.

OBSERVATIONS

A kind of mitochondrial crowding was observed in early spermatids of rabbits, stallions, dogs, and cats. The *rabbits* showed the most beautiful pictures. As soon as the spermatid was formed after the spermatocyte divisions, the mitochondria assembled in a dense, granular body near the nuclear membrane, surrounding the Golgi apparatus (Fig. 1). The body thus formed showed a sharp outline, as a rule, and only very rarely were a few isolated mitochondria

observed in the cytoplasm, probably representing an artifact. Later on, when the "archoplasmic vacuole" formed, the mitochondrial mass was pushed aside together with the Golgi apparatus, but it remained as a rule in the vicinity of the developing acrosome system, until the end of the spermatogenetic cycle, when one generation of mature spermatozoa went into the tubular lumen. Then the outlines of the dense mass became somewhat loosened, and generally many of the mitochondria spread in the cytoplasm (Fig. 2). At the beginning of the new cycle of spermatogenesis, the mitochondria were all found at some distance behind the elongated nucleus, which was moving into the deeper layers of the seminiferous epithelium (Fig. 3). Part of the mitochondrial body often remained in this state until the moment when the mitochondrial sheath of the middle piece began to form (Fig. 4), but in many spermatids no trace of the previous crowding was seen after the beginning of the new cycle of spermatogenesis. After the formation of the mitochondrial sheath, no mitochondria could be demonstrated with certainty in the cytoplasm of the spermatid, but the remnant of the Golgi apparatus was slightly stained, and granules of varying size, representing lipid droplets and particles rich in ribonucleic acid, often showed a deep colour.

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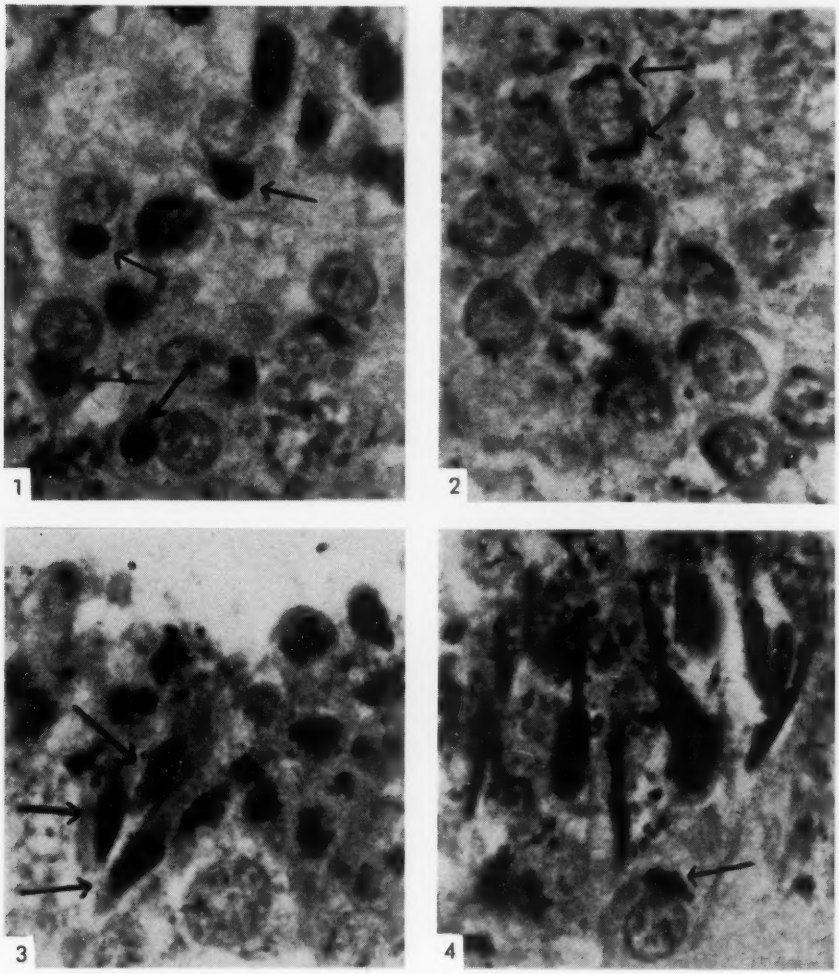
The young spermatids of *stallions* showed an accumulation of *most* of their mitochondria near or around the Golgi apparatus, but the mass formed was looser than in spermatids of rabbits, and no regular outline was present. The Golgi apparatus was rather intensely coloured by the mitochondrial stains, however, and thus a false impression of the size of the mitochondrial mass was often received. At the end of the spermatogenetic cycle all mitochondria spread in the cytoplasm, and later they occupied the long cytoplasmic lobe behind the transforming nucleus, except an area immediately surrounding the centrioles.

The mitochondria of young spermatids of *dogs* remained scattered during a short period and then formed a loose, rather large mass near the Golgi apparatus, but not surrounding it. At the end of the cycle the mass disintegrated, but on account of the rather scanty cytoplasm the mitochondria occurred even near the posterior pole of the elongated nucleus, contrary to the conditions in the other species studied.

The young spermatids of *cats* seldom showed the accumulation of all mitochondria into one group, but for a rather short period two or three groups formed near the nuclear membrane. They disintegrated before the end of the cycle.

No formation of distinct mitochondrial masses was observed in the spermatids of *bulls, rams,* or *mice* though there were often more mitochondria near the Golgi apparatus than in the remaining cytoplasm.

Young spermatids of *rats* and *guinea pigs* showed all mitochondria in the periphery of the cell, close to the cell membrane. This "margination" of the



LEGEND

The photomicrographs were taken from the same section.
Helly's fluid, aniline fuchsin-methyl green. 2000 ×

Fig. 1. Very early spermatids of rabbit. Four typical mitochondrial masses in contact with nuclei are marked by arrows.

Fig. 2. Spermatids of rabbit just before the start of a new spermatogenetic cycle. The mitochondrial masses have lost their regular shape and partly disintegrated. The arrows show two groups of mitochondria formed through division of the original large mass.

Fig. 3. A small area of the seminiferous epithelium of a rabbit, at an early stage of the cycle of spermatogenesis, with three elongated nuclei of spermatids (arrows). The remaining dark bodies are mitochondrial masses which are still rather dense, though their shape is irregular and peripheral disintegration is obvious.

Fig. 4. The upper two thirds show the formation of the mitochondrial sheaths of maturing spermatozoa of rabbit. No mitochondrial masses persist at this late stage of spermiogenesis. The lowest part of the photomicrograph shows an early spermatid with its mitochondrial mass (arrow) somewhat flattened and lifted from the nucleus by the developing archoplasmic vacuole.

mitochondria is known since the studies of REGAUD (1908) and DUESBERG (1910) and has been verified by electron microscopy (WATSON, 1952).

DISCUSSION

The mitochondria of the testes of rabbits have been described earlier by MASUI (1923) and GRESSON and ZLOTNIK (1945). According to MASUI the mitochondria of young spermatids are predominantly found in the *periphery* of the cell, as in guinea pigs. Later they form groups in the cytoplasm behind the elongated nucleus. GRESSON and ZLOTNIK (1945) observed a "subspherical body" in the young spermatids, but denied the mitochondrial nature of this body, which persisted in the cytoplasm cast off by the mature spermatozoa and thus did not take part in the formation of the middle piece. They reported scattered mitochondria in the cytoplasm of those spermatids containing "subspherical bodies". Their theory is that this body represents the archoplasm of the spermatid, which has been kept in a concentrated state within the Golgi apparatus and emerges from this structure very early in the development of the spermatid, before it resumes its original larger size. The material used in the present investigation did not show any mitochondria outside the large body, as a rule, and this body was formed by assembling mitochondria and later in the spermiogenesis it definitely disintegrated into many mitochondria, which entered into the formation of the middle piece. Nothing reminding of the mitochondrial body persisted in the residual cytoplasm cast off by the spermatozoa, though many lipid droplets and basophilic granules and the remnant of the Golgi apparatus often took mitochondrial stains and may have been mistaken for mitochondria by earlier authors.

The present description of mitochondrial accumulations, reminding of those seen in rabbits, in the spermatids of stallions is in agreement with MASUI (1919 a), whereas the presence of similar pictures in dogs has apparently not been reported before. On the other hand, GRESSON and ZLOTNIK (1945) observed a similar distribution of the mitochondria in spermatids of boars. MASUI (1919 a, b) stated that a "clotting" of mitochondria occurs immediately behind the elongated nucleus of the *late* spermatid of stallions and bulls, and that it reminds of the "nebenkern" of insects. The present study failed to produce any pictures reminding of those shown by MASUI. On the contrary, this region of the cytoplasm was generally devoid of mitochondria, except during a transitory stage of dog spermatids. The present observations on the distribution of mitochondria in cat spermatids are in agreement with LEPLAT (1910), and GRESSON and ZLOTNIK (1945). The last-mentioned authors also observed the diffuse distribution of the mitochondria in the spermatids of bulls and mice.

The dense mitochondrial body present in early spermatids of rabbits obvi-

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ously has a superficial resemblance to the early stages of the mitochondrial "nebenkern" of the spermatids of insects. However, it is only a transitory phenomenon, whereas the true "nebenkern" undergoes profound changes in its structure and eventually forms part of the spermatozoon. There seems to be some tendency in the mitochondria of young mammalian spermatids to accumulate around the Golgi apparatus. This tendency reaches its extreme in the rabbit, but is also evident in stallions and dogs, and is often suggested in others of the few species studied here. The "margination" of the mitochondria in the spermatids of rats och guines pigs is a remarkable contrast to the conditions observed in other mammals.

SUMMARY

All mitochondria of the young spermatids of rabbits assemble into a dense body reminding of the first stage in the development of the mitochondrial "nebenkern" of the spermatids of insects. A similar, but less pronounced arrangement is seen in stallions and dogs. These mitochondrial bodies disintegrate at a later stage of spermatogenesis and the mitochondria spread in the cytoplasm again before the middle piece is formed.

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ON THE CARDIAC PLEXUS OF THE VAGUS INTESTINALIS NERVES IN MYXINE*

BY

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1. INTRODUCTION

In cyclostomes the innervational system of the enteric canal is formed exclusively by the intestinal branches of the vagus nerves. In both Myxine (MÜLLER 1838, BRANDT 1922) and Petromyzon (JOHNELS 1956) the two nerves unite to form the ramus intestinalis impar which runs along the alimentary canal until close to the cloacal region. In Myxine the two intestinal branches of the vagus nerves branch repeatedly in the cardiac region (see RETZIUS 1826) and form a complicated plexus composed of a large number of nerves which interlace in a meshwork and gradually unite in a caudal direction and form the ramus intestinalis impar (MÜLLER 1838, BRANDT 1922, PIETSCHMANN 1933, HALLER v. HALLERSTEIN 1934, HIRT 1934, MARINELLI & STRENGER 1956). Such an intercalated plexus seems to be a rather unique feature. However, it has not been studied in detail previously.

2. METHODS

A number of 10—15 cm long specimens of Myxine glutinosa were collected at Kristineberg, on the west coast of Sweden. The fixatives were Bouin's fluid and Bodian's fixative No. 2 (ROMEIS 1948). Portions of the body comprising the caudal part of the branchial region and the anterior part of the trunk region were sectioned transversally and sagittally. The thickness of the sections was 10—15 microns. The staining was performed according to BODIAN (1936, see also ROMEIS 1948) or PALMGREN (to be published in this Journal).

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3. OBSERVATIONS

In the branchial region the trunks of the two vagus nerves run dorsally to the branchial sacs and laterally to the striated muscles of the branchial constrictor musculature. Behind the branchial region and approximately on a level with the ductus oesophageo-cutaneus the main branch of the vagus nerve destined to the oesophagus and the intestine on each side penetrates into the tissue of the strong striated muscles of the constrictor cardiae and reaches the wall of the oesophagus (as defined by MAAS 1899, see also COLE 1913). In this place the hepatic nerves—one or two on each side—branch from the main intestinal stems as well as a considerable number of branches which innervate the cardiac constrictor musculature. Of these muscular nerve branches one is particularly large and originates from the left vagus nerve. It is destined to the sphincter musculature around the ductus oesophageo-cutaneus which also receives a number of fibres from the right vagus.

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It is only natural that the strong musculature of the cardiac constrictor has a large number of nerve branches which serve the innervation. The splitting up of the vagus intestinalis nerves into a plexus is, on the other hand, more puzzling. The nerves of the plexus pass through the region of the constrictor muscle and it seems unlikely that the formation of the plexus can be connected with the innervational system of the cardiac constrictor. The plexus is formed by mostly rather thick nerves in the same way as the brachial and lumbosacral plexuses of higher vertebrates. These plexus nerves are destined to form the ramus intestinalis impar and it must be particularly stressed that this plexus exhibits no similarity to the terminal nervous plexus of the enteric canal.

In the anterior half of the region of the cardiac constrictor muscles the nerves of the plexus are mostly located on the interior side of and very close to the striated muscles. The space between the striated constrictor muscles and the sparse smooth muscular layer of the oesophagus wall is filled with loose connective tissue in which the nerves are embedded. However, many of the plexus nerves penetrate into the musculature of the cardiac constrictor (see fig. 2 c) and run for shorter or longer distances within the muscular tissue of the constrictor cardiae. In the posterior half of the extension of the cardiac constrictor the arrangement of the muscle fibres is less regular, the muscle fibres becoming more sparse in a caudal direction. In this portion the nerves attain a superficial position in relation to the scattered striated muscle fibres.

Within the tissue of the muscle of the constrictor the shape of the nerve stems seems to be very dependent on the space left for them in the surrounding muscle bundles. The nerves in the plexus are flattened towards the interior surface of the striated musculature or between the bundles of muscles in conformity with the main direction of the muscular elements, which is circular

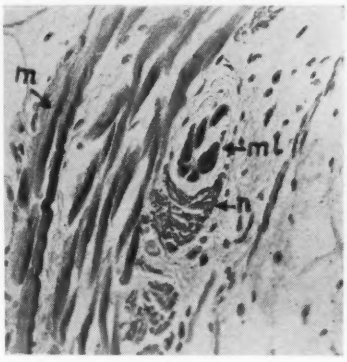

Fig. 1. Transverse section of oesephagus wall of Myxine. m. — circular layer of constrictor cardiae. ml — longitudinal muscle bundle of constrictor cardiae. n. — branch in plexus of right vagus intestinalis. Note the shape of this particular nerve as compared with the shape of nerves demonstrated in fig 2 a and c. Microphotograph 175 ×.

(see fig. 2 a, c). In some places where longitudinal muscular bundles are present the shape of the nerves is strongly influenced in a different way (see fig. 1). It is evident that of the two elements, the muscles and the nerves, the latter appear to be more plastic, as seen in the sections, and to take their shape from the muscles.

Generally speaking the course of the nerves of the plexus is mainly in a caudal direction. Naturally the splitting up of the nerves into the meshes of the plexus causes deviations from this main direction. But in addition to this the nerves often make very sharp bends which are mostly almost perpendicular to the longitudinal axis of the intestine. These bends may turn around a muscle bundle into or out of the musculature of the constrictor cardiae (fig. 2) but the nerves outside the muscular tissue also have similar sharp turns which, thus, do not appear to be related to any kind of tissue obstacles. These bends are repeated at usually very short intervals and do not cause any permanent change in the main direction of the nerves which, consequently, appear strongly wavy in their course. This wavy appearance seems to be typical of the intestinal as well as the muscular nerves within the coat of the striated cardiac constrictor muscle and, accordingly, the nerves of the plexus are considerably longer than the distance between the anterior limit of the cardiac constrictor muscle and the point of union of the nerves into the ramus intestinalis impar caudally to the constrictor muscle.

The nerves of Myxine generally seem to have a wavy course. But in the small portions of the nerves which are more or less strictly oriented perpendicularly to the longitudinal axis of the intestine the very loose and wavy appearance of the fibrous elements is particularly striking (fig. 2 b). It seems that the connective tissue elements which keep the fibres of the nerves together are also exceedingly loosely arranged in these particular portions.

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Fig. 2. From transverse sections of oesophagus wall of Myxine. m. — musculature of the constrictor cardiae. n. — one of the large branches of plexus of the left vagus intestinalis. Smooth musculature close to the letter n in the pictures. a. is situated 80 microns in front of b., which is 100 microns in front of c. Note the wavy structure of nerve fibres of the sharp bend sectioned in b. Before the bend the nerve is situated inside muscular layer of the constrictor (a). After the bend the nerve is within the musculature and is split up into two branches (c). Oesophagal epithelium in the left lower corner of the pictures. Note ganglion cells (g.c.) in b. Microphotographs, 190 ×.

4. DISCUSSION

Evidently the cardiac constrictor musculature is a specialized part of the striated branchial constrictor. The bundles of this muscle have been added to the wall of the short postbranchial oesophagus in the vicinity of the place where the intestinal branches from the vagus first reach the wall of the intestinal canal. In any case this idea explains why, in this region, the nerves change position in relation to the layer of striated muscles and have to pass inside a strong sphincter musculature.

The function of the constrictor cardiae is to stop the entrance of the branchial water current into the intestine. Thus the muscle has to be tightly constricted whenever the animals do not feed. On the other hand this portion of the intestinal canal has to be strongly widened when food is passed into the oesophagus (see MARINELLI & STRENGER 1956). It is evident that the activity of the constrictor muscles implies considerable dislocations of the structures involved. The wavy arrangement of the nerves allows of a participation in the contractions and distensions of the oesophagus in spite of the fact that the nerves themselves are only very slightly elastic. Thus, the particular relations between the muscular and nervous elements which have been described above may be explained by the necessity of mutual mobility between the two elements. It seems also probable that the continuous function of the nerves in passing through the normally tightly contracted sphincter is favoured by the division of the large vagus stems into smaller units. Thus it seems probable that the peculiar plexiform structure of the anterior portions of the vagus intestinalis branches evolved at the same time as the cardiac constrictor muscle became perfected.

5. SUMMARY

Some characteristic features of the plexiform structure of the vagal branches in the cardiac region of the alimentary canal in Myxine are described. It is concluded that the musculus constrictor cardiae evolved as a caudal extension of the branchial constrictor musculature, which may explain the fact that the nerve stems of the vagus run laterally to the musculature in the branchial region but medially of the striated muscular coat of the cardiac constrictor. The plexiform shape of the proximal portions of the vagus intestinalis branches probably evolved simultaneously with this cardiac sphincter musculature as a response to mechanical influence from this musculature.

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